Remarks

Claims 1, 2, 8, 11, 15, 16, 18, 19, 21-35, 37, 42, 43, 45-47, 50, 52-54, 56, 60, 62, 68, 70, 76, 77, and 83-89 were under consideration in the office action. Claims 11, 15, 16, 18, 19, 21, 23 and 26-34, 37, 42, 53 and 83 have been withdrawn from consideration. The examiner has rejected claims 1, 2, 8, 22, 24, 25, 35, 43, 45-47, 50, 52, 54, 56, 62, 68, 70, 76-77 and 84-89. The office action summary also indicated claim 60 was rejected, although no grounds of rejection are stated in the office action. The present amendment adds claims 90 and 91. Upon entry of the amendments, claims 1, 2, 8, 11, 15, 16, 18, 19, 21-35, 37, 42, 43, 45-47, 50, 52-54, 56, 60, 62, 68, 70, 76, 77, and 83-91 are pending.

Upon further review, the applicants note that claim 8 was mistakenly identified as reading on the elected species.

The examiner states that claims 11, 15, 18, 19, 21, and 23-34 were withdrawn as being drawn to a non-elected *invention*. These claims, together with claim 83, are drawn to the elected *invention*. Claims 11, 15, 18, 19, 21, 23, 26-34 and 83 do not, however, read on the elected *species*. These claims are subject to rejoinder when the patentability of an allowable generic claim to that encompasses the elected species is established. It is pointed out that claims 24 and 25 read on a process of making the elected species and that the examiner in fact examined these claims. It is believed that claims 24 and 25 were included in the examiner's listing of withdrawn claims in error. In the above listing and attached claim listing, claims 24 and claims 25 are therefore not indicated as withdrawn.

Claims 90 and 91 are added herein and read on the elected species.

Restriction Requirement

The maintenance of the restriction requirement is acknowledged. Further reconsideration is respectfully requested.

In considering applicants' traversal the examiner asserts that "the fact that rings A and B can be different itself points out to lack of the same or the corresponding technical feature."

The WIPO Preliminary Examination Guidelines, which provide authoritative guidance upon the interpretation of the unity of invention standard under the P.C.T. (the standard applicable in an application filed under 35 U.S.C. § 371) explicitly contradict the examiner's assertion. The WIPO Preliminary Examination Guidelines explicitly point out, "the fact that the alternatives of a Markush group can be differently classified is not, taken alone, considered to be justification for a finding of lack of unity of invention". P.C.T. International Search and Preliminary Examination Guidelines, 10.17(d) (March 25, 2004). Even if the options for A and B are capable of being differently classified as either carbocyclic or heterocyclic aromatic rings does not change the fact that they are aromatic rings. Since the examiner has not found a reference that prevents the novel structures of Formula I being considered a special technical feature linking the claims, the examiner has failed to set forth any valid grounds for the finding of lack of unity.

The applicant also respectfully requests reconsideration of the lack of unity finding as to the conjugate claims of Group III. As the applicants pointed out in traversing the rejection, the novel structures of Formula I (whether the rings A or B are carbocyclic or heterocyclic) are a structural feature of the conjugates claimed in claims 37, 42 and 53. The conjugates claimed in claims 37, 42 and 53 contain all the structural features of the compounds of Formula I together with a link to an antibody. The examiner has not provided any reasons why the compounds of Formula I should not be considered a unifying feature linking the claims to the compounds of Formula I with the conjugates of Group III. The structure of Formula I (as claimed in claim 1) is literally linked to and therefore present as a feature of the conjugates of Group III. Even if the examiner were correct in finding two separate groups of Formula I compounds (carbocyclic and heterocyclic), there would be no basis for asserting disunity between each group of Formula I compounds and the corresponding antibody conjugates.

Based upon the foregoing it is respectfully submitted that the examiner should find unity of invention as to Groups I, II and III and therefore that the restriction requirement should be withdrawn.

Explanation of the Amendments

Claims 90 and 91 are added and are directed to the species of claim 2 or 35 wherein a is 2 or 3. The added claims supported by the recitation in claim 2 that a is 1, 2, or 3.

Claim 8 is amended to depend from claim 90.

Claims 1, 2, 8, 18, and 84 to 89 are amended to correct "$-(C_2-C_6)-OR^4$" to "$-(C_2-C_6)$<u>alkylene</u>-OR^4" and "$-O(C_2-C_6)-N((C_1-C_6)alkyl)_2$," to $-O(C_2-C_6)$<u>alkylene</u>-$N((C_1-C_6)alkyl)_2$. In addition, in claims 1, 2, and 84 to 89 the list of substituents for which substituents for substituted aryl and heterocyclic groups is amended to eliminate those substituents where substituted aryl and substituted heterocyclic groups are not included within the substituent definition.

Claim 8 is amended to depend from claim 90.

Response to the Rejections

(1) Rejection of Claims 43, 45-47, 50, 52, 54, 56, 62, 68, 70, 76-77 and 84-89 under 35 U.S.C. § 112 First Paragraph Enablement Requirement.

The examiner has rejected claims 43, 45-47, 50, 52, 54, 56, 62, 68, 70, 76-77 and 84-89 under 35 U.S.C. § 112 as allegedly containing subject matter which is not described in the specification in such a way as to enable a person skilled in the art to make and or use the invention. The applicants respectfully traverse.

The examiner concludes on the basis of several factual assertions that the claims are insufficiently enabled. The examiner states the state of the prior art requires *in vitro* and *in vivo* screening to determine which compounds exhibit the desired pharmacological properties, there is

no absolute predictability, that the pharmaceutical art is unpredictable, and the more unpredictable the art, the more specific enablement is needed. The breath of the claims is treating an individual with the compounds as defined in claim 43. The examiner states that the quantity of experimentation needed would be undue because the person skilled in the art would need to determine what kinds of proliferative disease could be treated by the administration of the compounds. The examiner also states that the level of skill in the art is high, but, even so, every embodiment of the invention would have to be tested *in vitro* and *in vivo* to determine which compounds have the desired activity and which diseases could be treated.

Applicants enjoy a presumption that the specification, which discloses how to make and use the claimed invention, complies with the first paragraph of 35 U.S.C. § 112, unless there is a reason to doubt the objective truth of the specification. *See In re Marzocchi*, 439 F.2d 220 (CCPA 1971). The initial burden of establishing a basis for denying patentability to a claimed invention therefore rests upon the examiner. *See In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988); *In re Thorpe*, 777 F.2d 695 (Fed. Cir. 1985); *In re Piasecki*, 745 F.2d 1468 (Fed. Cir. 1984).

An application satisfies the enablement requirement if the disclosure has sufficient information to enable the person skilled in the pertinent art to make and use the claimed invention without undue experimentation. *In re Wands*, 858 F.2d 731, 737, (Fed. Cir. 1988). The fact that experimentation may be required and may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), *aff'd. on other grounds sub nom., Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985). See also *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404. The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504, (C.C.P.A. 1976).

The factors to be considered in determining whether any necessary experimentation is "undue" include the factors described in *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988),

namely: (1) the breadth of the claims; (2) the nature of the invention; (3) the state of the prior art; (4) the level of one of ordinary skill; (5) The level of predictability in the art; (6) the amount of direction provided by the inventor; (7) the existence of working examples; and (8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

The examiner's analysis is couched in terms of an analysis under the *Wands* factors. The gist of the examiner's grounds of rejection appears to be the contention that the treatment of cancer and other proliferative disorders lacks "absolute predictability even in view of the seemingly high level of skill in the art". The examiner states that each embodiment would therefore be required to be individually tested for physiological activity. The examiner is apparently of the view that *in vivo* and *in vitro* testing would be required for every embodiment to determine which embodiments would be effective. The examiner states that in a more unpredictable area, "more specific enablement" is needed to satisfy the statute (citing *In re Fisher*, 427 F.2d 833 (C.C.P.A. 1970)).

The applicants respectfully point out that the examiner has not met the evidentiary burden required to overcome the presumption that applicants have met the enablement requirement is met. The examiner has made various assertions concerning the state of the art, but no evidence has been produced, and no reasons have been presented why the evidence would cause the person skilled in the art to question objectively whether the person skilled in the art would be able to practice make and use the invention recited in the rejected claims.

It is moreover respectfully submitted that the examiner overstates both the level of unpredictability and the level of validation needed in order to satisfy the enablement requirement of 35 U.S.C. § 112 first paragraph. At the same time the examiner underestimates the level of skill and state of knowledge in the art, as well as the amount of experimentation typically involved in drug development. All these factors show that the quantity of experimentation required to practice the claimed invention would be far from undue.

As correctly pointed out by the examiner, the claims are directed to the treatment of proliferative diseases by administering 2-arylpropenoic acid arylamides. The compounds are cytotoxic to abnormally proliferating cells but also protective of normally proliferating cells. The rejected claims are directed both to treatment of proliferative diseases by administering the compounds to kill abnormally proliferating cells as well as using the compound cytoprotectively in the presence of another agent (a cytotoxic agent or radiation) which kills the abnormally proliferating cells.

The nature of the invention claimed in the rejected claims is that the rejected claims are all directed, in one way or another, to the treatment of diseases characterized by abnormal cell proliferation, particularly cancer. A common mechanism underlies all the diseases, namely abnormal cell proliferation.

The level of skill in the art is acknowledged to be very high. The persons skilled in the art will typically be Ph.D. scientists specializing in drug discovery and development and/or medical doctors specializing in cancer treatment therapies.

With regard to the state of the art and level of predictability, the art is not as unpredictable as characterized by the examiner. For example, researchers have identified the cell cycle as being crucial in diseases involving abnormal cell proliferation, such as cancer, making it credible that agents that intervene appropriately in the cell cycle could "halt[a] cancer cell in its tracks". See K. Collins, *et al., Proc. Nat. Acad. Sci. USA*, **1997**, *94*, 2776-78, a copy of which is provided herewith. Consistent with this, such anticancer drugs as are available tend to be effective against a broad range of cancers. The applicants provide herewith drug data sheets for some of the commonly used anticancer drugs, cisplatin, doxorubicin, and paclitaxel obtained from the Cancer Care Ontario website (http://www.cancercare.on.ca/index_drugFormulary.htm). Each drug is shown as being effective versus a range of cancers. For example, cisplatin has established utility versus at least the following cancers: bladder cancer, ovarian cancer, testicular cancer, brain tumors, cervical cancer, germ cell tumors, head and neck cancer, both small cell

and non-small cell lung cancers, neuroblastoma, osterosarcoma, esophageal cancer, Wilm's tumor, adrenocortical cancer, breast cancer, endometrial cancer, gastrointestinal cancer, gynecological sarcoma, hapatoblastoma, malignant melanoma, non-Hodgkin's lymphoma and thyroid cancer. Although the documents supplied are the current versions of the documents, it is respectfully submitted that since these drugs are well-established cancer drugs that the information can be taken as reflecting approximately the same state of the art as existed at the time of filing of the present application.

The examiner characterizes the state of the art as unpredictable. While it may be true that the treatment of cancer, for example, is unpredictable, it is also true that broadly useful anticancer treatments are available. Such unpredictability as exists in the treatment of cancer can be largely explained due to the nature of the disease, particularly the variability in individual cancers and factors such as variability in the aggressiveness of the cancer, metastasis, and development of drug resistance. While these factors make response in between individuals variable and somewhat unpredictable, they do not detract from the general usefulness of a given drug.

In addition, with regard to the state of the art, routine *in vitro* screening methods are available to discover drugs that are effective in the treatment of proliferative disorders. For example, the applicants cite and provide data for in vitro cytotoxicity assays versus cancer cell lines. Such screening assays have been shown to correlate with in vitro activity and clinical effectiveness of cancer drugs. Rose, *et al.*, showed a significant correlation between *in vitro* and *in vivo* assays when fermentation extracts evaluated in an *in vitro* cycotoxicity assay against several tumor cell lines and then tested *in vivo* against P388 leukemia or B16 melanoma. W.C. Rose, *et al.*, "Correlation of in vitro cytotoxicity with preclinical in vivo antitumor activity", *Anticancer Res.*, **1988**, *8*(3), 355-67. Additional evidence of the predictiveness of *in vitro* cell line models was provided in a study by Voskoglou-Nomikos, *et al.* who showed that in vitro cytotoxicity results were predictive of efficacy observed in Phase II clinical trials in a study of thirty one anti-cancer compounds. T. Voskoglou-Nomikos, *et al.*, "Clinical Predictive Value of

the in Vitro Cell Line, Human Xenograft, and Mouse Allograft Preclinical Cancer Models", *Clinical Cancer Res.*, **2003**, *9*, 4227-39. Copies of the Rose and Voskoglou-Nomikos references cited are supplied herewith.

The *in vitro* screening methods described in the preceding paragraph can be carried out routinely. One very prominent example of the routineness of assessment of new chemical entities for activity in proliferative disorders is the *In Vitro* Cell Line Screening Project (operated by the National Cancer Institute (NCI) of the National Institutes of Health for anticancer drug discovery. The NCI program has the capacity to screen 3,000 compounds per year against 60 different human tumor cell lines. A copy of a printout from the NCI website describing the program is provided herewith.

The applicants have provided considerable guidance and working examples for carrying out the invention. The specification provides detailed information defining the compounds of the invention as well as defining preferred embodiments of the compounds and describing methods of making the embodiments. Considerable guidance is also provided for the formulation and administration of the compounds as well as for administering the compounds and carrying out the claimed methods of the invention. For example methods of screening for cytotoxic activity versus four cancer cell lines (Example 15), induction of apoptosis in tumor cells (Example 16), Radioprotection (Examples 17-21), and protection of normal cells from the cytotoxic effects of cytotoxic agents (Examples 22-26) are described.

In view of the foregoing factors, the amount of experimentation required to practice the invention cannot be described as undue.

The compounds of the invention would be readily synthesized using conventional techniques for organic chemical synthesis. The examiner will note that the compounds of the invention are amides which are particularly easily synthesized, for example through high throughput synthesis techniques.

The compounds of the invention could then be subjected to routine *in vitro* screening using the techniques described in the specification and otherwise known in the art. As the example of the NCI's screening program (with a capacity to test 3,000 compounds per year in 60 different cell lines) shows, such *in vitro* screens could be very routinely performed. As shown by the references cited by the applicants, such *in vitro* screens are predictive of *in vivo* activity. Thus it would not be necessary to perform both *in vitro* and *in vivo* tests for every embodiment of the inventention. Rather, the *in vitro* screening results would be predictive of *in vivo* activity, and therefore results from the *in vitro* screening results could be used to predict which compounds would be most effective *in vivo*.

The *in vitro* screens can also be used to predict activity across a range of diseases. As shown above, anticancer drugs tend to be effective across a range of cancers, and thus, effectiveness of an agent in treating one proliferative disorder would be predictive of activity against another proliferative disorder. This arises because of the common mechanism - abnormal cell proliferation involving the cell cycle - involved in the different diseases. The same is true with regard to the methods involving cytoprotection.

The data presented in the specification for applicants' compounds predict of consistent activity across a range of proliferative diseases. In Table 5 (pp. 100-101), the applicants present data for ten diverse compounds within the scope of the invention that were tested against four cell lines from four different types of cancer. The results showed remarkably consistent activity for any given compound across the different cell types, i.e. the most potent compounds (Examples 1, 6, 10 and 11) were equally potent across all four cell types.

Even to the extent that activity is not consistent across different diseases, the person skilled in the art who wished to use the invention to treat a particular disease could readily implement a suitable *in vitro* screen by selecting a suitable cell line. The person skilled in the art would be able to choose a cell line characteristic of those which are proliferating abnormally in the particular disease condition from the very wide array of cell lines that are available. For

example, data for cytotoxicty assays for four different cell lines, representing four different cancers are given in the specification in Example 15. However, the NCI screen uses at least 60 cell lines, and many further cell lines are described in the literature.

The examiner states that there is no "absolute predictability". The applicants agree, and therefore concede that it is possible that not every compound within the scope of the invention will be useful for every claimed utility within the scope of the rejected claims. "Absolute predictability" is not, however, a prerequisite for enablement. Were "absolute predictability" the standard for enablement, few, if any, patents would issue, particularly in the chemical, biochemical and pharmaceutical arts. The courts have recognized that the level of validation required for patentability is lower than required, for example, to obtain F.D.A. approval to market a new drug. *See, e.g.*, *In re Brana*, 51 F.3d , 1560, 1568 (Fed. Cir. 1995) ("Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development."). The need for further experimentation is therefore fully consistent with the enablement requirement. *In re Wands*, 858 F.2d 731, 736 (Fed. Cir. 1988). The fact that the claims may encompass inoperative embodiments is insufficient to show non-enablement, and the claims therefore need not exclude all inoperative embodiments. See *Atlas Powder Co. v. E.I. Du Pont De Nemours & Co.*, 750 F.2d 1569, 1576, (Fed. Cir. 1984) (citing *In re Dinh-Nguyen*, 492 F.2d 856, 858-59 (C.C.P.A. 1974)).

In view of the complexity of developing the field of developing new pharmaceuticals, the quantity of experimentation required to practice the invention of the rejected claims cannot be described as undue. *Wands* recognized that the need for further experimentation is not inconsistent with enablement: "the key word is undue not experimentation". *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988) (quoting *In re Angstadt*, 537 F.2d 498, 504, 190 (C.C.P.A. 1976) (internal quotation marks omitted). Pharmaceutical drug discovery and development, and the treatment of cancer and other proliferative disorders are complex, to be sure, but the law does not preclude inventions in complex fields from patent protection. It is recognized that the fact that experimentation may be required and may be complex does not necessarily make it undue, if the

art typically engages in such experimentation. MPEP 2164.01 (citing *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983)). Few fields of endeavor rival the complexity of developing pharmaceuticals. It is respectfully submitted that the fact of the very high cost associated with developing a new drug (estimated to be in the range from about \$500 million to \$2,000 million for each new chemical entity) is reflective of the fact that drug companies "typically engage in" a substantial amount of experimentation in the course of drug development. The amount of experimentation that would be required to practice the claimed invention would therefore not be "undue".

In view of the foregoing, reconsideration of the examiner's findings concerning enablement of claims 43, 45-47, 50, 52, 54, 56,. 62, 68, 70, 76-77 and 84-89 is respectfully requested. It is respectfully submitted that upon such reconsideration, the examiner should conclude that the rejection for lack of enablement under 35 U.S.C. § 112 should be withdrawn.

(2) Rejection of Claims 1, 2, 8, 22 and 35.

The examiner has rejected claims 1, 2, 8, 22 and 35 under 35 U.S.C. § 103(a) as allegedly unpatentable for obviousness over Yamamori, *et al.*, JP2001-139550 ("Yamamori"). The applicants respectfully traverse.

The examiner states that Yamamori teaches structurally similar compounds to those claimed in the rejected claims and compounds on page 15, particularly Ia-64, Ia-65 and I-63. The examiner states that the reference's compounds are similar to the claimed compounds, but that "most of the compounds made in the reference has been proviso out in herein". The examiner states, however, that it would have been prima facie obvious for the person of ordinary skill in the art to make compounds within the generic disclosure of the reference because they are allegedly similar to the compound claimed in the rejected claims with a reasonable expectation of achieving a composition for use in the method described in the reference. The examiner states that compounds Ia-68 and Ia-64 teach the equivalence of OH and OMe.

The examiner claims to have established a *prima facie* case of obviousness. As set forth in the MPEP, three basic criteria must be met to establish a *prima facie* case of obviousness,

(1) A suggestion or motivation to modify the reference or to combine reference teachings.

(2) A reasonable expectation of success.

(3) The reference (or references when combined) must teach or suggest all the claim limitations.

MPEP 2142. As the MPEP further notes, "[t]he teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicants' disclosure. *Id.* (citing *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991)).

The applicants respectfully point out the examiner has failed to establish a *prima facie* case of obviousness. In the first instance, it is not clear which reference the examiner is citing in support of the obviousness rejection - whether it is the abstract or the underlying patent. If the examiner wishes to rely on the patent, the examiner is respectfully reminded that the MPEP requires the examiner to provide a translation of the document. MPEP 706.02.

Despite this lack of clarity, it is respectfully submitted that it is nevertheless clear that the examiner not established a *prima facie* case of obviousness. A *prima facie* case of obviousness must, in addition to showing the differences between the prior art reference establish the reasons that the person skilled in the art would be motivated to modify the teachings of the reference to provide the claimed invention.

The abstract of the reference demonstrates that an enormous diversity of compounds fall within the generic formula, i.e. formula I, which would not have obviously led to the presently claimed invention. True, it appears from the abstract that Ar^1 and A can be phenyl which can be substituted, Z *can be* O, R, Y1 and Y2 *can be* hydrogen, and n *can be* 1. However, the generic disclosure of the Yamamori abstract encompasses, in addition to these possibilities, an extremely large number of other possibilities when all possible combinations disclosed in the abstract are

taken into account. It is clear that the examiner has used the applicants' teachings to make the appropriate selections in the generic formula set forth by Yamamori. However, the use of such hindsight is not appropriate to establish why the invention presently claimed *would have been* obvious to the person skilled in the art at the time the presently claimed invention was made. The MPEP makes clear that in order for *prima facie* obviousness must be established based on the reference itself unguided by the applicants' disclosure, i.e. obviousness is determined looking forward from the reference not backward from the claimed invention.

Looking to the Yamamori patent itself, insofar as it is understood by the applicants, the examiner has not stated a reason it would have been obvious to modify the compounds taught in the reference to make compounds within the scope of the rejected claims. It appears to the applicants that sufficient information may be deduced from the Japanese version of the document to demonstrate convincingly that the invention claimed in the rejected claims would not have been obvious in view of the reference. The applicants therefore wish to set forth their assumptions concerning the content of the untranslated Japanese patent. It is to be understood that since the examiner has not provided a translation of the document, these assumptions are not to be taken as facts established by the examiner to support the rejection of the claims for non-obviousness. Neither are any of the assumptions to be considered as facts admitted by the applicants. Rather, the applicants wish to make a good faith effort to advance prosecution by responding as fully as possible to the examiner's asserted grounds of rejection.

The assumptions made by the applicants are as follows. The applicants assume that the examiner considers that the compounds in the tables on pages 14-26 were compounds made by Yamamori, *et al.*, with the intent that they would have potential utility in treating arteriosclerosis. Some screening data appear to be presented on p. 38. Applicants assume that the data are from a screen that is somehow relevant to arteriosclerosis, and therefore a lower MED figure would indicate a more potent compound. Applicants also assume that Yamamori, would have been more motivated to make useful compounds than less useful compounds. Therefore it is assumed that the features which occur most frequently in the compounds in the tables on pages 14-26 of

the reference indicate the features that were found to be most beneficial in compounds for treating. Conversely, features that are absent or occur with low frequency are assumed to be features that were either found not to be beneficial by Yamamori, or which were found to be detrimental to the anti-arteriosclerosis activity.

Making these reasonable assumptions, it is clear that even without the benefit of a translation of Yamamori the presently claimed invention can be established to be non-obvious over Yamamori.

As defined in claim 1 of the present application, the essential features of the compounds of formula I include: (1) with regard to ring A, that it be other than pyridyl, quinazolyl, or naphthyridyl and at least monosubstituted; (2) with regard to ring B, that it be at least disubstituted, with the additional proviso that when ring B is phenyl, the substituents are other than 2,3-di-(OR^4) or 3,4-di-(OR^4).

These essential features of the compounds of claim 1 separately appear infrequently in the compounds described in the reference, and do not appear together at all in any of the compounds. Focusing principally upon the propenamide-type compounds in the reference (i.e. compounds **Ia**) as being most closely related to the presently claimed compounds, approximately 160 of the 209 Yamamori **Ia** compounds have Ar^1=pyridyl, a feature which is excluded from the scope of claim 1. In addition, 193 of the 209 compounds Yamamori **Ia** have unsubstituted Ar^1, a feature which is excluded from the scope of claim 1 by the requirement that the corresponding ring be at least monosubstituted. In the Yamamori **Ia** compound, ring A need not be aromatic. However, of the approximately 195 Yamamori **Ia** compounds where ring A is aromatic, approximately 140 compounds have ring A which is unsubstituted or monosubstituted aromatic. Of the approximately 45 Yamamori **Ia** compounds where ring A is aromatic and at least disubstituted, about 40 are phenyl that is 2,3- or 3,4-disubstituted with oxygenated substituents (OH, OMe, OAc, etc.), a feature which is also excluded from the scope of claim 1 either because

the substituents do not fall within the scope of the definition of R^2, or due to the proviso excluding 2,3-di-(OR^4) and 3,4-di-(OR^4).

Considering the Yamamori reference as a whole, it is respectfully submitted that the person skilled in the art who wished to make more compounds with similar properties to those described by Yamamori would make compounds that had the features that are represented most frequently in the compounds that Yamamori made, as these would be assumed to be the features that impart the desired effectiveness to the compounds for treating arteriosclerosis. For example, it appears from Yamamori that for Ar^1, unsubstituted rings, particularly 2-pyridyl are strongly preferred, while for A, unsubstituted or monosubstituted rings are preferred. The person skilled in the art would therefore be led to make compounds with these features rather than features which would lead to compounds within the scope of the rejected claims. The data given on p. 38 reinforce this conclusion, showing that compound Ia-39 (having Ar^1=2-pyridyl and A=4-chlorophenyl) is the most potent compound for which data are presented.

The examiner points to compounds having some of the features of the instantly claims, particularly Ia-64, Ia-65 and "I-63". The applicants do not note a compound "I-63" - it is assumed that the examiner is referring, perhaps, to compound Ia-63. If the examiner is suggesting that it would be obvious for the person skilled in the art to modify these compounds to prepare compounds within the scope of the instant claims, the examiner has failed to state why the person skilled in the art would have been drawn to these particular compounds from rather than the four hundred, or so, other compounds described in the reference, nor has the examiner articulated the particular modification that it would be obvious for the person skilled in the art to make, or why such modification would be made. The examiner states that compounds Ia-68 and Ia-64 teach the expressly teach the equivalence of OH and OMe. However, it is not seen that Ia-68 and Ia-64 in fact make any such teaching (the compounds differ in more than replacement of OH by OMe, or vice versa, plus no equivalent effect is shown). Neither is it seen what relevance such teaching would have in establishing the obviousness of the presently claimed invention.

Clearly the examiner's attention is drawn to the particular compounds identified due to the similarity the examiner perceives between the identified compounds and those presently claimed. However, the fact there may be similarity between isolated compounds in the reference and the presently claimed compounds when the reference's compounds are viewed with knowledge of the presently claimed invention does not demonstrate why subject matter of the presently claimed invention as a whole *would have been* obvious at the time the invention was made. In order to establish a *prima facie* case of obviousness, MPEP 2142 requires that reasons be established why the person skilled in the art would have been led to make the changes required to the subject matter of the reference to yield the claimed invention. The examiner has not provided such reasons.

Based on the foregoing, the examiner has failed to establish a *prima facie* case of obviousness of claim 1 or its dependent claims. If the examiner is relying on the abstract only, the examiner has not presented reasons that the person skilled in the art would be led to select compounds within the scope of claim 1 from the multitude of structural possibilities represented by the abstract. If the examiner is relying on the underlying document, the examiner has not provided a translation as required under MPEP 706.02 nor articulated reasons why the person skilled in the art would be led to modify the reference. It is incumbent on the examiner to provide reasons why the person skilled in the art would modify the reference to provide the claimed invention in order to establish a *prima facie* case of obviousness.

The applicants also respectfully point out the rejected dependent claims have features that even further distinguish these claims from the reference. Claim 8 requires a 3-nitrogenous substituent as R^{3m}. Claim 22 is drawn to particular compounds. The examiner has not identified which compound or compounds within the scope of claim 22 are specifically rendered obvious by the reference and the reason the person skilled in the art would have been led by the reference to prepare a particular compound within the scope of claim 22. Claims 90 and 91 added herein require ring A to be at least disubstituted.

It is respectfully submitted that since the examiner has failed to establish a *prima facie* case of obviousness of claims 1, 2, 8, 22, and 35, the rejection of the claims under 35 U.S.C. § 103(a) should be withdrawn.

(3) Rejection of Claims 24-25.

The examiner has rejected claims 24-25 under 35 U.S.C. § 103(a) as allegedly unpatentable for obviousness over Abraham, *et al.*, U.S. Pat. No. 5,705,521 ("Abraham"). The applicants respectfully traverse.

The examiner states that Abraham teaches a similar process of preparing structurally similar compounds. The examiner states it would have been *prima facie* obvious to a person of ordinary skill in the art to use the process of Abraham by using similar starting materials as claimed in the rejected claims because the process per se is an analogous process.

It is respectfully pointed out that in making the rejection, the examiner here has made the same error that was made by the examiner in *In re Ochiai*, 71 F.3d 1565 (Fed. Cir. 1995) (discussed in MPEP 2116.01). The examiner has relied on a *per se* rule that a process claim is obvious if a prior art reference discloses an "analogous process" using similar starting materials. In *Ochiai*, the Federal Circuit stated: "the examiner incorrectly drew from *Durden* ... a general obviousness rule: namely, that a process claim is obvious if the prior art references disclose the same general process using 'similar' starting materials. No such per se rule exists." *Ochiai*, 71 F.3d at 1570 (citation omitted). The court further made clear that a claimed process is not rendered obvious by the disclosure of an "analogous process" using "similar" starting materials in the prior art unless the prior art suggests the desirability of making such a modification. *Id.* (quoting *In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984)) ("The mere chemical possibility that one of those prior art acids could be modified such that its use would lead to the particular cephem recited in claim 6 does not make the process recited in claim 6 obvious 'unless the prior art suggested the desirability of [such a] modification.'").

As set forth in the MPEP, three basic criteria must be met to establish a *prima facie* case of obviousness:

(1) A suggestion or motivation to modify the reference or to combine reference teachings.

(2) A reasonable expectation of success.

(3) The reference (or references when combined) must teach or suggest all the claim limitations.

MPEP 2142. As the MPEP further notes, "[t]he teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicants' disclosure. *Id.* (citing *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991)).

The applicants respectfully point out the examiner has failed to establish a *prima facie* case of obviousness. In claims 24 and 25, applicants are clearly not claiming a novel synthetic method for making amides, but rather are claiming a method of making particular amides using particular starting materials as defined in claims 24 and 25. The examiner concedes that the cited reference, Abraham, does not disclose describe the synthesis claimed in claims 24 and 25. Therefore in order to establish a *prima facie* case of obviousness MPEP 2142 (as well as the law of obviousness as explained in *Ochiai*) requires the examiner to establish the reasons why a person skilled in the art would modify the Abraham reference to perform the methods of claims 24 and 25. Abraham itself does not appear to suggest making such a modification, and the examiner has not cited any other reference in the rejection of claims 24 and 25 to establish a reason for making such a modification.

It is respectfully submitted that since the examiner has failed to establish a *prima facie* case of obviousness of claims 24-25 that the rejection of the claims under 35 U.S.C. § 103(a) should be withdrawn.

(4) Rejection of Claim 60

The office action summary lists claim 60 as being rejected. The applicants do not however find any rejection of claim 60 in the office action, and no grounds for the rejection of claim 60 are stated. The examiner is respectfully requested to clarify whether in fact claim 60 was in fact rejected, and, if so, to state the grounds for the rejection, if such grounds remain in view of applicants' remarks to rejections of other claims as set forth above.

Conclusion

Based on the foregoing, all claims are believed in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,

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The cell cycle and cancer

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Recent insights in the fields of cell cycle regulation and cancer would each alone have provided prime examples of research at the "Frontiers of Science." However, some of the most revealing information about both topics has derived from the intersection of the two fields. The intent of this summary is to introduce the basics of the cell cycle, cancer, and their overlap, and then to describe the research from two laboratories that was presented in the session. A more comprehensive treatment of these subjects, beyond this description for a general audience, is contained in several reviews (1–5).

The process of replicating DNA and dividing a cell can be described as a series of coordinated events that compose a "cell division cycle," illustrated for mammalian cells in Fig. 1 (see legend for details). At least two types of cell cycle control mechanisms are recognized: a cascade of protein phosphorylations that relay a cell from one stage to the next and a set of checkpoints that monitor completion of critical events and delay progression to the next stage if necessary. The first type of control involves a highly regulated kinase family (2). Kinase activation generally requires association with a second subunit that is transiently expressed at the appropriate period of the cell cycle; the periodic "cyclin" subunit associates with its partner "cyclin-dependent kinase" (CDK) to create an active complex with unique substrate specificity. Regulatory phosphorylation and dephosphorylation fine-tune the activity of CDK–cyclin complexes, ensuring well-delineated transitions between cell cycle stages. In the future, additional molecular definition of the cell cycle may lead to a more intricate progression than indicated in Fig. 1.

A second type of cell cycle regulation, checkpoint control, is more supervisory. It is not an essential part of the cycle progression machinery. Cell cycle checkpoints sense flaws in critical events such as DNA replication and chromosome segregation (4). When checkpoints are activated, for example by underreplicated or damaged DNA, signals are relayed to the cell cycle-progression machinery. These signals cause a delay in cycle progression, until the danger of mutation has been averted. Because checkpoint function is not required in every cell cycle, the extent of checkpoint function is not as obvious as that of components integral to the process, such as CDKs.

Superficially, the connection between the cell cycle and cancer is obvious: cell cycle machinery controls cell proliferation, and cancer is a disease of inappropriate cell proliferation. Fundamentally, all cancers permit the existence of too many cells. However, this cell number excess is linked in a vicious cycle with a reduction in sensitivity to signals that normally tell a cell to adhere, differentiate, or die. This combination of altered properties increases the difficulty of deciphering which changes are primarily responsible for causing cancer.

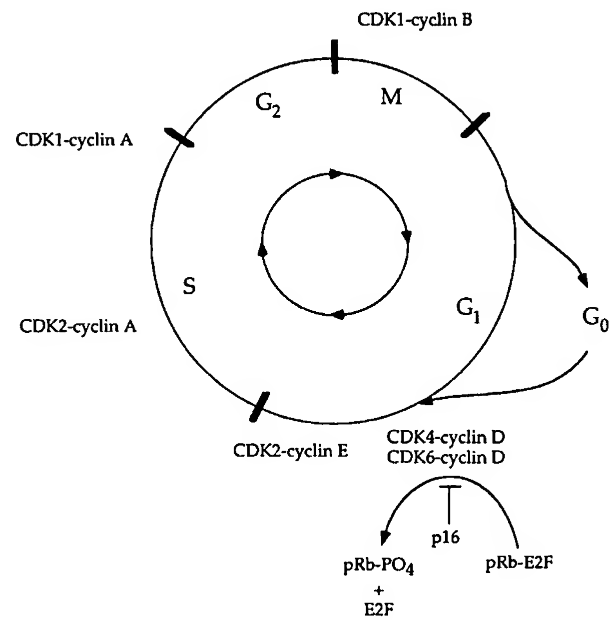

FIG. 1. A schematic representation of the mammalian cell cycle. In each cell division cycle, chromosomes are replicated once (DNA synthesis or S-phase) and segregated to create two genetically identical daughter cells (mitosis or M-phase). These events are spaced by intervals of growth and reorganization (gap phases G_1 and G_2). Cells can stop cycling after division, entering a state of quiescence (G_0). Commitment to traverse an entire cycle is made in late G_1. Progress through the cycle is accomplished in part by the regulated activity of numerous CDK–cyclin complexes, indicated here and described in the text.

The first genetic alterations shown to contribute to cancer development were gain-of-function mutations (6). These mutations define a set of "oncogenes" that are mutant versions of normal cellular "protooncogenes." The products of protooncogenes function in signal transduction pathways that promote cell proliferation. However, transformation by individual oncogenes can be redundant (mutation of one of several genes will lead to transformation) or can be cell type-specific (mutations will transform some cells but have no effect on others). This suggests that multiple, distinct pathways of genetic alteration lead to cancer, but that not all pathways have the same role in each cell type.

More recently, the significance of loss-of-function mutations in carcinogenesis has become increasingly apparent (7). Mutations in these so-called "tumor suppressor" genes were

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Abbreviation: CDK, cyclin-dependent kinase.

initially recognized to have a major role in inherited cancer susceptibility. Because inactivation of both copies of a tumor suppressor gene is required for loss of function, individuals heterozygous for mutations at the locus are phenotypically normal. Thus, unlike gain-of-function mutations, loss-of-function tumor suppressor mutations can be carried in the gene pool with no direct deleterious consequence. However, individuals heterozygous for tumor suppressor mutations are more likely to develop cancer, because only one mutational event is required to prevent synthesis of any functional gene product.

It now appears that tumor suppressor gene mutations are highly likely to promote, and may even be required for, a large number of spontaneous as well as hereditary forms of cancer (5). But what are the functions of tumor suppressor gene products in a normal cell? Although this is a topic for future research, there is suggestive evidence that several tumor suppressor genes encode proteins that negatively regulate cell cycle progression. Loss of function of the tumor suppressor gene product pRb, for example, would be predicted to liberate E2F transcriptional activators without requiring phosphorylation and thus bypass a normal negative regulation controlling entry into the cycle (Fig. 1). Loss of the tumor suppressor gene product p16 would have a similar consequence, liberating E2Fs by increasing pRb phosphorylation (Fig. 1). In addition, cell cycle progression can be halted at several points by the tumor suppressor gene product p53, activated in response to checkpoints sensing DNA and possibly also chromosome damage; loss of p53 would remove this brake to cycling (8).

By what molecular pathway does loss of cell cycle regulation in an organism lead to cancer? What genetic changes can cooperate to accomplish the cancer cell's escape from the normal balance of cell growth? Tyler Jacks described results from his laboratory that addressed these questions, using mice and cell lines derived from mice that have been engineered to lack individual tumor suppressor gene products. To create "knock-out" mice, embryonic stem cells that can later be introduced back into a developing animal are subject to targeted mutagenesis of the gene of interest. Cells with one mutant gene copy are injected into early embryos, and mice that use the injected cells to form germ-line tissue are selected for breeding. Some progeny will be entirely heterozygous for the mutant gene; these mice can then be bred to obtain homozygous mutant animals.

One important insight from the studies of mice lacking tumor suppressor genes is the dependence of balanced cell numbers on not only the regulation of cell proliferation but also on the regulation of cell death. In the past, cell death was regarded as an accidental failure of normal cell function. However, often the opposite is true: genetic studies of cell death indicate a requirement for active death signals and directed execution (for review of proteins involved in cell death see ref. 9). One collection of experiments illustrates the significance of combining genetic alterations that deregulate both cell proliferation and cell death (ref. 10; see also refs. 11 and 12). Inactivation of pRb during embryogenesis promotes inappropriate cell cycle activity. This follows from the role of pRb in negatively regulating entry into the cell cycle (Fig. 1). In contrast to expectations, however, the increased cell cycle activity in Rb null mice does not result in a net increase in cell number. This is due to a commensurate increase in cell death that specifically eliminates the abnormally cycling cells. This cell death is often dependent on the function of p53, as demonstrated from the analysis of RB/p53 double-mutant embryos.

The function of p53 in sentencing inappropriately growing cells to death has implications for cancer development and chemotherapy. Murine tumors with functional p53 respond to chemotherapy by promoting their own demise, but those lacking p53 typically do not (13). A balance between cell proliferation and death likely functions during development to create a finely patterned body map. This normal function of the cell death pathway and the potential for tipping the balance too much toward death in some degenerative diseases will be exciting future topics of investigation.

Clearly, the products of cell cycle regulatory genes are critical determinants of cancer progression. But precisely how do gene sequence alterations and missing regulatory components affect the functioning of the cell cycle machinery? Having in hand molecular details of the protein structures would address this question and would also suggest strategies for cancer therapy. Nikola Pavletich described research in his laboratory that has yielded high-resolution structures of p53 and of inactive and active states of CDK2. These structures were determined from the x-ray diffraction patterns of purified, crystallized proteins.

Although p53 may serve many roles in the cell, its best-characterized function is as a transcriptional activator. The residues of p53 that are frequently mutated in cancer cells are critical for DNA binding (14). A p53–DNA co-crystal structure revealed that these frequently mutated residues fold together into one region of the surface of the protein (15). Thus, cancer-promoting mutations that occur throughout the primary sequence of the protein are in fact clustered in one functional domain.

Recent studies have focused on the structural basis for regulation of the CDKs, using CDK2 as a model system (for review of CDK regulatory mechanisms see ref. 2). In mammalian cells, CDK2 functions in S-phase with cyclin A as a partner (Fig. 1). The association of cyclin A modifies the previously determined CDK2 structure (16) by reorienting a catalytically critical glutamic acid into the catalytic cleft and moving away the regulatory loop that can block access of a protein substrate to bound ATP (17). Cyclin A binding stimulates CDK2 activity, but phosphorylation of threonine-160 is required for full activation. The crystal structure of threonine-phosphorylated CDK2 complexed with cyclin A reveals conformational change in the substrate-binding site and also a strengthening of CDK2–cyclin A interaction (18).

Finally, one mechanism for the inactivation of the CDK2–cyclin A complex was examined: binding of the inhibitor p27 (19). Co-crystals of CDK2–cyclin A with the N-terminal inhibitory domain of p27 reveal that bound p27 physically blocks the active site, inserting itself into the catalytic cleft. Also, p27 association modifies the structure of the "roof" of the ATP-binding site and blocks a putative protein substrate docking region on cyclin A. With these structural modifications in mind, it may be possible to design small molecules that will have the same effect: blocking CDK activity, thus halting the cancer cell cycle in its tracks.

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A DRUG NAME: CISPLATIN

SYNONYM(S): Cis-platinum, cis-diamminedichloroplatinum, CDDP, DDP, CIS, Platinum

COMMON TRADE NAME(S): Cisplatin (Mayne)

B MECHANISM OF ACTION AND PHARMACOKINETICS

Cisplatin was first synthesized in 1845, but its cytotoxic properties were not described until 1965. Cisplatin entered into clinical trials in 1971. Cisplatin is an inorganic complex formed by an atom of platinum surrounded by chlorine and ammonia atoms in the *cis* position of a horizontal plane. Intracellularly, water displaces the chloride to form highly reactive charged platinum complexes. These complexes inhibit DNA through covalent binding leading to intrastrand, interstrand, and protein cross-linking of DNA, leading to apoptosis. Experimental and clinical data suggest that cisplatin enhances radiation therapy effects.

Oral Absorption	No	
Distribution	Well distributed with highest levels in kidney, liver and prostate; found in breast milk, distributes into third spaces such as ascites and pleural fluid, crosses placenta.	
	Cross blood brain barrier?	Trace
	PPB	Cisplatin: not significantly Platinum: 90%
Metabolism	Non-enzymatically transformed to multiple metabolites	
	Active metabolite(s)	Yes (free, filterable platinum)
	Inactive metabolite(s)	Yes
Excretion	Primarily in urine (90%); undergoes renal secretion as well as excretion. Platinum present in tissues for up to 180 days	
	Urine	10-40%(platinum)
	t ½	Cisplatin: 30 minutes Free complexes: ≥ 5 days

C INDICATIONS AND STATUS

- * Bladder cancer (neoadjuvant, adjuvant or palliative)
- * Ovarian cancer (first or second line)
- * Testicular cancer (single agent or combination)
- * Brain tumours (pediatric)
- * Cervical cancer
- * Germ cell tumours
- * Head and neck cancer
- * Lung cancer, small cell; non-small cell
- * Neuroblastoma (pediatric)
- * Osteosarcoma (pediatric)
- * Esophageal cancer
- * Wilm's (pediatric)

Other uses include:

Adrenocortical cancer
Breast cancer
Endometrial cancer
Gastrointestinal cancer
Gynecological sarcoma
Hepatoblastoma
Malignant melanoma
Non-Hodgkin's lymphoma
Thyroid cancer

*** Health Canada approved indication***

D ADVERSE EFFECTS

ORGAN SITE	SIDE EFFECT	ONSET		
Cardiovascular	Bradycardia , LBBB (rare)	I	E	
	Hypertension	I	E	
	Cerebral arteritis, CVA (rare)		E	
Neurologic	Hearing loss, tinnitus (31%), vertigo			D
	Autonomic neuropathy (rare)			D
	Seizures (rare)			D
	Dorsal column myelopathy (rare)			D
	Hiccups		E	
	Peripheral neuropathy			D
	Optic neuritis, blurred vision, altered colour perception			D
	Acute encephalopathy (rare)		E	D
***Extravasation hazard* (refer to Appendix 2)**	**IRRITANT**		I	
Gastrointestinal	Taste abnormality			
	Nausea and vomiting		E	I
	Diarrhea			
	Anorexia			

D

ADVERSE EFFECTS (Continued)

ORGAN SITE	SIDE EFFECT	ONSET
Hematologic	myelosuppression, (25-30% nadir 18-23 days)	E
	Anemia	E
	Hemolytic anemia (Coombs positive)	
	Thrombotic microangiopathy (rare)	E
Neoplastic	Acute leukemia	L
Dermatologic	Alopecia	E
	Rash	I E
Hepatic	Elevated liver function tests, including bilirubin (transient, rare)	E
Hypersensitivity	Type I (anaphylactic), (1-20%)	I
	Type II (hemolytic anemia)	E
Renal/metabolic	Toxic nephropathy (28-36%)	E
	Hypomagnesemia, hypocalcemia, hypokalemia, hyponatremia, hypophosphatemia	E
	Hyperuricemia	E
	SIADH	E
Reproductive	Infertility	L
Other	Muscle cramps	
	Increased plasma iron levels	
	Raynaud's syndrome (rare)	D

Dose-limiting side effects are underlined.
I = immediate (onset in hours to days); E = early (days to weeks);
d = delayed (weeks to months); L = late (months to years)

The major dose limiting toxicity of cisplatin is cumulative nephrotoxicity. Tubular necrosis of both proximal and distal renal tubules occurs. Although reversible, effects are cumulative and occur in 28-36% of patients treated with a single dose of $50mg/m^2$. Renal toxicity but may be permanent with high doses or prolonged treatment. ***Nephrotoxicity*** can be minimized or prevented by IV hydration and mannitol diuresis. Furosemide diuresis is theoretically hazardous due to potential additive ototoxicity. Serum creatinine concentration is an inadequate indicator of nephrotoxicity in children. Nuclear medicine GFR measurement is preferred, when this test is available.

D ADVERSE EFFECTS (Continued)

Renal tubular abnormality such as acidosis, hypomagnesemia, hypophosphatemia, hyponatremia or hypokalemia may be present with normal glomerular function. ***Hypomagnesemia (magnesium wasting)*** may become severe enough to cause tetany. Hypomagnesemia may persist for greater than one year following treatment. Children are particularly at risk.

Cisplatin produces moderate to severe ***nausea and vomiting*** in virtually all patients. Nausea and vomiting may start within one hour and last up to 24 hours. Tolerance improves with IV hydration and 5-day continuous infusion. Various degrees of nausea and anorexia may persist for up to 1 week, even with well-controlled acute nausea and vomiting. The use of prophylactic and continuing antiemetic medication is recommended.
(See Supportive Care & Symptom Control Regimens)

Neurotoxicity consists of peripheral neuropathy, which is sensory in nature but can also include motor effects, reduced deep-tendon reflexes and loss of proprioception. Symptoms usually occur after prolonged therapy (4-7 months) and may be irreversible. Seizures, altered taste, slurred speech, and memory loss has occurred rarely. Cisplatin should be discontinued if functionally important neuropathy develops. ***Ototoxicities*** consist of audiogram abnormalities in 24% of patients. Auditory impairment usually results in impairment of acuity in the high frequency range, but may affect the normal hearing range in 6% of cases. Ototoxicity is cumulative, dose-related and irreversible. Ototoxicity appears to be related to peak levels of cisplatin, as significant hearing loss has been reported with single high doses. Ototoxicity may be more severe in children. Cranial irradiation may lower the cumulative dose at which cisplatin will cause hearing loss. Vestibular ototoxicity is rare.

Anaphylactic reactions with tachycardia, hypotension, erythema, wheezing and facial edema should be treated with antihistamines and hydrocortisone (Solu-Cortef®). For future doses, pretreat with the same medications. ***Anemia*** can result from prolonged cisplatin therapy and should be corrected with transfusion of packed red blood ***cells*** to maintain hemoglobin of greater than 90 g/L. Cisplatin-induced anemia has been shown to respond to erythropoietin.

E DOSING

Refer to protocol by which patient is being treated. Numerous dosing schedules exist and depend on disease, response and concomitant therapy. Guidelines for dosing also include consideration of white blood cell count. Dosage may be reduced and/or delayed in patients with bone marrow depression due to cytotoxic/radiation therapy. All patients should receive adequate hydration protocols and premedication for emesis.

Adults:

Infusion: Q3-4 w: 20mg/m^2 IV for 5 days OR
50-100mg/m^2 day 1

Dosage in myelosuppression: modify according to protocol by which patient is being treated; if no guidelines available, refer to **Appendix 6** "Dosage Modification for Myelosuppression"

Dosage with renal impairment See specific protocol. In general renal function should have normalised before patients are retreated. The use of cisplatin should be avoided if creatinine clearance is < 50mls/min. If cisplatin is used in the presence of impaired renal function, reduced doses should be used, for example (AHFS DI 2002):

CrCl 10-50mls/min:	give 75% of dose
CrCl < 10mls/min:	give 50% of dose, or discontinue

Dosage with hepatic impairment: no adjustment required

E DOSING (Continued)

Children:

Dosage and safety not definitively established.

Infusion:	Q1week:	$30 mg/m^2$
	Q3 week:	$90 mg/m^2$
Dosage in renal impairment:	The usual practice in children is to withhold cisplatin if the GFR is less than 60 $mL/min/1.73\ m^2$.	

F ADMINISTRATION GUIDELINES (see Appendix 3a)

- Ensure good urinary output during chemotherapy visit; Patient should void at least once during chemotherapy visit
- Blood pressure should be taken before and after chemotherapy
- Additional hydration may be ordered for hypovolemic patients
- Hydration and diuresis for patients with pre-existing renal, cardiac, or diabetic history at discretion of physician
- Oral hydration with 8 glasses of fluid per day (for 1-2 days) is strongly encouraged; if nausea and vomiting prevent oral hydration, the patient may need to return for more IV hydration

Sample Hydration Protocols:

Dose	Prehydration	Infusion	Post-hydration
< $50mg/m^2$	*250mls NS over 30 min* ***OR*** *500mL NS over 60 min*	*Infuse in 100-250mls NS over 15-60 min* ***May** add 10G Mannitol with Cisplatin*	*100-250mls NS* ***OR*** *500mls NS with 10Meq KCL*
≥ $50mg/m^2$ * in-patient	***OR*** *500-1000mL NS with 10 Meq KCl over 2 hours* ***May** add 20-40mg of furosemide or 10G mannitol* ***May** add 20-40mg furosemide*	*Infuse in 250-500mL NS over 60minutes Give 50G mannitol (concurrently with cisplatin or split with pre and post hydration) unless furosemide given with prehydration*	*1000mls NS with 20 meq KCL (2g Magnesium Sulfate may also be added) over 1 hour* ***May** give magnesium glucoheptonate (100mg/ml) 30ml PO QID x 4 days*

* For in-patient, may pre-hydrate with 1-2L (NS, 2/3:1/3) over 8-12 hours; post-hydrate with 1-1.5L of IV fluid.

G SPECIAL PRECAUTIONS

Cisplatin is physically incompatible with any I.V. set, needle or syringe containing aluminium. Cisplatin is ***contraindicated*** in patients with known hypersensitivity to platinum containing compounds. ***Administer with caution*** to individuals with pre-existing renal impairment, myelosuppression or hearing impairment.

Cisplatin is ***mutagenic and carcinogenic and potentially fetotoxic,*** crosses the placenta and should not be used in ***pregnancy***. Present in ***breast milk***, therefore, breast feeding is not recommended.

Amifostine may be indicated for amelioration of certain toxicities of cisplatin (see **CCO Practice Guidelines:** Use of Amifostine to Ameliorate the Toxic Effects of Chemotherapy in the Treatment of Cancer)

All patients should receive appropriate hydration and anti-emetic protocols according to local guidelines.

H INTERACTIONS

AGENT	EFFECT	MECHANISM	MANAGEMENT
Renally excreted drugs (especially ifosfamide, high dose methotrexate, amphotericin, bleomycin)	decreased renal clearance and increased t½; toxicities of these drugs may be enhanced	reduced renal function caused by cisplatin	ascertain renal function prior to giving potentially toxic renally-excreted drugs (such as other chemotherapy) and modify doses as necessary
Aminoglycosides amphotericin	increased nephrotoxicity	additive effects	avoid or use with extreme caution during or shortly after cisplatin therapy
Etoposide	synergistic effect against certain tumours when combined with cisplatin (testicular cancer, lung cancer)	possibly by decreased clearance of etoposide	some protocols are designed to take advantage of this effect
Pyridoxine (high dose > 300mg/m^2)	reduced efficacy when given with cisplatin *and* altretamine	unknown	avoid concomitant use with the combination of cisplatin and altretamine

H INTERACTIONS (Continued)

AGENT	EFFECT	MECHANISM	MANAGEMENT
Furosemide Ethacrynic acid	increased ototoxicity	additive	avoid concomitant use; use furosemide if a diuretic is essential (may be less ototoxic than ethacrynic acid)
Paclitaxel (given after cisplatin)	increased toxicity and reduced efficacy if given after cisplatin	reduced clearance of paclitaxel results in increased toxicity (neutropenia). Reduced efficacy of paclitaxel because of reduced cycling of cells.	give paclitaxel prior to cisplatin when used in combination
Phenytoin	decreased phenytoin serum levels	decreased absorption and/or increased metabolism of phenytoin	monitor phenytoin serum levels; increase phenytoin dose if necessary

I RECOMMENDED CLINICAL MONITORING

Recommended Clinical Monitoring

- Baseline and regular renal function tests
- Hearing & Neurologic toxicity ratings each visit
- Serum magnesium, sodium, potassium, phosphate and calcium levels with each dose or cycle
- Baseline and regular CBC
- Audiogram after cumulative dose of 360mg/m^2

Suggested Clinical Monitoring

- Baseline & Periodic audiograms
- Baseline and regular liver function tests

J REFERENCES

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A

DRUG NAME: DOXORUBICIN

SYNONYM(S): Hydroxyl daunorubicin, Dox, Adria

COMMON TRADE NAME(S): Adriamycin®, Adriamycin PFS®, Adriamycin RDF® (Pfizer Canada) Doxorubicin (Mayne, Novopharm)

B

MECHANISM OF ACTION AND PHARMACOKINETICS

Daunorubicin and its 14-hydroxy derivative, doxorubicin, are anthracycline antibiotics produced by the fungus streptomyces peucetius. Doxorubicin damages DNA by intercalation of the anthracycline portion, metal ion chelation, or by generation of free radicals. Doxorubicin has also been shown to inhibit DNA topoisomerase II which is critical to DNA function. Cytotoxic activity is cell cycle phase-nonspecific.

Oral Absorption	No (5%)	
Distribution	Highest concentrations in liver, spleen, kidney, heart, small intestines, lung; crosses placenta; found in breast milk	
	Cross blood brain barrier?	No
	Vd	25 L/kg
	PPB	79-85%
Metabolism	Liver (major site) and other tissues; elimination primarily via liver and biliary system. Clearance is reduced, with elevated levels of doxorubicin and its metabolites, in patients with hepatic dysfunction especially if bilirubin elevated.	
	Active metabolite(s)	Doxorubicinol (major metabolite)
	Inactive metabolite(s)	Yes
Excretion	Predominantly in bile, 40-50% in feces within 7 days	
	Urine	4-5% over 5 days
	t ½ ∝	12 minutes
	t ½ ß	3.3 hours
	t ½ γ	29.6 hours

C INDICATIONS AND STATUS

- * Acute lymphocytic leukemia
- * Acute myeloblastic leukemia
- * Bladder cancer (intravenous and intravesical)
- * Breast cancer
- * Endometrial cancer
- * Gastric cancer
- * Head and neck cancer, squamous cell
- * Hodgkin's disease
- * Small cell lung cancer
- * Non small cell lung cancer
- * Neuroblastoma
- * Non-Hodgkin's lymphoma
- * Osteogenic sarcoma
- * Ovarian and gynaecologic cancer
- * Sarcoma, soft tissue
- * Testicular cancer
- * Thyroid cancer
- * Wilms' tumour

* ***Health Canada approved indication***

Other uses include:

Adrenocortical cancer
Carcinoid syndrome (small bowel)
Ewing's sarcoma
Gynecological sarcoma
Hepatic cancer
Islet cell cancer
Multiple myeloma
Pancreatic cancer
Prostate cancer
Retinoblastoma
Rhabdomyosarcoma

D ADVERSE EFFECTS

ORGAN SITE	SIDE EFFECT	ONSET			
Cardiovascular	Transient arrhythmia, bundle branch /AV block, ST changes (41%)	I			
	Pericarditis/myocarditis		E	D	
	Congestive heart failure			D	L
	Thromboembolism /thrombophlebitis			D	L
Dermatologic	Facial flushing with rapid injection	I			
	Photosensitivity	I	E		
	Urticaria				
	Radiation recall reaction (rare)	I			
	Alopecia (complete in most patients)		E		
	Hyperpigmentation of skin, mucosa nails (in children, rare)			D	
Extravasation hazard (refer to Appendix 2)	**VESICANT**, local necrosis	I	E		

D

ADVERSE EFFECTS (Continued)

ORGAN SITE	SIDE EFFECT	ONSET			
Gastrointestinal	Nausea and vomiting	I			
	Anorexia	I	E		
	Diarrhea ; typhlitis	I	E		
	Mucositis (stomatitis, esophagitis) (may occur 5-10 days after)		E		
Hematologic	Myelosuppression (primarily leukocytes), nadir 6-13 days, recovery in 21-24 days		E		
Neoplastic	Secondary leukemia				L
Hypersensitivity	Type I (anaphylactic), (rare)	I			
	Skin rash, fever, chills		E		
Injection site	Doxorubicin flare (histamine release)	I			
	Pain on injection	I			
	Chemical phlebitis	I			
Ocular	Conjunctivitis/lacrimation (rare)		E		
Renal/metabolic	Red colouration of urine for 1-2 days	I			
	Increased transaminases		E	D	
	Hyperuricemia (during periods of active cell lysis)	I			
Reproductive	Amenorrhea, hot flashes				
	Infertility				L

Dose-limiting side effects are underlined.
I = immediate (onset in hours to days); E = early (days to weeks);
D = delayed (weeks to months); L = late (months to years)

D ADVERSE EFFECTS (Continued)

Myelosuppression is the most common dose limiting toxicity; severe and fatal infections may occur.

Hyperuricemia during periods of active cell lysis, which is caused by cytotoxic chemotherapy of highly proliferative tumours of massive burden (e.g., some leukemias and lymphomas), can be minimized with allopurinol and hydration. In hospitalized patients the urine may be alkalinized, by addition of sodium bicarbonate to the IV fluids, if tumour lysis is expected.

Cardiotoxicity may manifest as initial acute effect with transient electrocardiographic abnormalities, reported in up to 41% of patients; and a later cumulative, dose-dependent cardiomyopathy. The acute electrocardiographic changes are usually reversible, unrelated to total dose, return to baseline readings within a few days to two months and usually are not an indication to discontinue the doxorubicin. Acute life threatening arrhythmias have been reported rarely.

The more serious cardiotoxicity is a dose-dependent cardiomyopathy (0.4-9% of all patients), which has an attendant mortality as high as 61%. The onset of cardiomyopathy may be delayed, occurring 6 months or more after therapy. The incidence of drug-induced congestive heart failure at cumulative doses of 300 mg/m^2 is 1-2% in contrast to 30% incidence with cumulative dose >550 mg/m^2. For a graphic estimate of the cumulative probability of developing doxorubicin-induced congestive heart failure (CHF) in adults versus total cumulative dose, see Von Hoff 1979. Certain patients (prior mediastinal radiation, prior anthracyclines, older age, hypertension) are at higher risk and may develop cardiotoxicity at lower cumulative doses of doxorubicin and should receive cumulative doses of doxorubicin <400mg/m^2. In adults with risk factors cardiac function monitoring (echocardiogram or MUGA scan) should be performed before treatment and periodically throughout treatment. All patients who have received total cumulative doses of 450 mg/m^2 and in whom further therapy with doxorubicin is indicated should undergo cardiac assessment before continuing treatment. The risk of cardiotoxicity may be lower with weekly regimens; total cumulative doses should not exceed 700mg/m^2 even with weekly regimens. Dexrazoxane may be use as a cardioprotectant in patients with advanced or metastatic cancer who are at risk of developing cardiotoxicity when receiving chemotherapy containing doxorubicin. See **CCO Practice Guidelines** "Use of Dexrazoxane as a Cardioprotectant in Patients Receiving Doxorubicin or Epirubicin Chemotherapy for the Treatment of Cancer"

Children less than 15 years of age are more likely to develop CHF from cumulative doses greater than 550 mg/m^2 than those patients aged 15 to 40 years. In children, clinical cardiotoxicity increases rapidly at a cumulative dose of about 450 mg/m^2, but individual patients may have a lower threshold and develop toxicity at a significantly lower dose. Cardiac monitoring should be done a minimum of alternate courses, and at each course with doses > 300mg/m^2. Cardiac dysfunction may appear several months to years after anthracycline therapy, therefore monitoring should continue after therapy is complete.

Erythematous streaking (a histamine release phenomena) along the vein proximal to the site of injection has been reported, and must be differentiated from an extravasation event. This ***'doxorubicin flare'*** reaction usually subsides within 30 minutes. The injection may be continued, more slowly in the same site or may be changed to another site. Diphenhydramine 25 mg (1 mg/kg/dose in children), or hydrocortisone 100 mg (1 mg/kg/dose in children), by slow IV push over 5 minutes into the IV line may hasten clearing of the reaction.

The ***tissue necrosis*** that occurs with ***extravasation*** may happen days to weeks after the treatment. Patients must be observed for delayed reactions and prior injection sites carefully inspected.

Doxorubicin has the potential to enhance radiation injury to tissues. While often called ***'radiation recall reactions'***, the timing of the radiation may be before, concurrent with or even after the administration of the doxorubicin. The skin is the site most commonly affected, resulting in erythema followed by dry desquamation. Skin reactions generally occur only if the drug is given within 7 days of the radiation. Rarely, reactions after 30 days have been noted. Skin involvement, while unpleasant, is not as debilitating as is the case for internal organs. Enhancement of radiation injury to the esophagus and gastrointestinal tract is most severe when the drug and the radiation are given concomitantly. Recurrent injury to a previously irradiated site may occur weeks to months following radiation.

Intravesical use may result in pain, hematuria and reduced bladder capacity.

E DOSING

Refer to protocol by which patient is being treated. Numerous dosing schedules exist and depend on disease, response and concomitant therapy. Guidelines for dosing also include consideration of white blood cell count. Dosage may be reduced and/or delayed in patients with bone marrow depression due to cytotoxic/radiation therapy, or tumour infiltration, and in the elderly. Frequently administered in combination with other cytotoxics.

Intravenous:
q1w: 10-20 mg/m^2 bolus
q3-4w: 60-75 mg/m^2 bolus
q4w: 20-30 mg/m^2/day bolus for 3 consecutive days

Intravesical:
q1w: 50-80 mg via bladder instillation (in 50-100mls), retained 1-2 hours, weekly x 4 then monthly. Voided urine should be inactivated with hypochlorite solution.

Maximum lifetime dose:

	3 weekly regimen	*Weekly regimen*
Cardiac risk factors	400 mg/m^2	550 mg/m^2
No cardiac risk factor	550 mg/m^2	700 mg/m^2

Dosage in myelosuppression: modify according to protocol by which patient is being treated; if no guidelines available, refer to **Appendix 6** "Dosage Modification for Myelosuppression"

Dosage with renal impairment: no adjustment required

Dosage with hepatic impairment: Doxorubicin is contraindicated in patients with severe hepatic impairment, especially with elevated bilirubin. Consideration should be given to dose modification for patients with severe increases in transaminases; limited data exists on the use of doxorubicin in this setting as these patients are routinely excluded from clinical trials.

Bilirubin (μmol/L)	% usual dose
1-2x ULN	50%
2-4x ULN	25%
>4x ULN	0%

Dosage in the elderly: Use with caution

Dosage in obesity: Systemic clearance lower, use with caution.

F ADMINISTRATION GUIDELINES (see Appendix 3a)

- Slow push through sidearm of free flowing IV (0.9% Sodium Chloride Injection or 5% Dextrose, Normal Saline or 2/3.1/3) Give 2 to 4mg (1-2ml) per minute.
- Doses ≤100mg may be mixed in 50mL minibag (5% Dextrose), doses >100mg may be mixed in 100mL minibag (5% Dextrose); Infuse through sidearm of free flowing IV over 10-30 minutes.
- Do not admix with other drugs unless data are available; precipitates with fluorouracil and heparin
- Slow down injection rate if erythematous streaking occurs.
- If any signs or symptoms of extravasation occur, the injection or infusion should be immediately terminated and restarted in another vein.
- **PROTECT FROM LIGHT.**

G SPECIAL PRECAUTIONS

Cardiac toxicity is cumulative across members of the anthracycline (doxorubicin, epirubicin, daunorubicin, idarubicin and anthracenedione (mitoxantrone) class of drugs). Patients who have received these agents are at increased risk of toxicity, and should be carefully monitored. The cumulative doses resulting in cardiotoxicity are lower in patients who have received radiation to the mediastinal area of concomitant therapy with other cardiotoxic agents such as cyclophosphamide.

Contraindicated in patients with severe cardiovascular disease, cardiac failure, unstable conditions including recent myocardial infarction, hypertension, angina and arrhythmias; persistent myelosuppression induced by previous treatment with other antineoplastic agents or by radiotherapy; in patients with hyperbilirubinemia or severe hepatic impairment, in patients who have exceeded the maximum cumulative doses of agents in this class, or in patients with hypersensitivity to doxorubicin, its excipients, or other anthracyclines or anthracenediones.

Contraindicated for intravesical use in patients with bladder infections, inflammation or with invasive tumours.

Doxorubicin is ***mutagenic, genotoxic, embryotoxic and carcinogenic*** and should not be used in ***pregnancy***. Its effects on fertility have not been fully established but does lead to infertility which may be partially reversible. Present in ***breast milk***, therefore breast feeding is not recommended.

H INTERACTIONS

AGENT	EFFECT	MECHANISM	MANAGEMENT
Barbiturates	therapeutic effects of doxorubicin decreased	increased plasma clearance of doxorubicin	monitor if barbiturates initiated or discontinued
Cyclophosphamide	exacerbation of cyclophosphamide induced hemorrhagic cystitis	uncertain	caution
Cyclophosphamide	increased risk potential for cardiotoxicity	uncertain	monitor, may need to modify dose of doxorubicin
Digoxin	decreased digoxin levels; interaction may occur several days after treatment	decreased digoxin absorption	monitor digoxin levels and patient
Mercaptopurine	enhanced hepatotoxicity	uncertain	monitor
Quinolones	antimicrobial effect of quinolones decreased	decreased quinolones absorption	monitor, may need to modify dose of quinolones

H

INTERACTIONS (Continued)

AGENT	EFFECT	MECHANISM	MANAGEMENT
Cytarabine	Typhlitis	uncertain, treat appropriately	
Streptozocin	increased toxicity of doxorubicin	liver damage by streptozocin decreases metabolism of doxorubicin	caution
Zidovudine	decreased effect of zidovudine	doxorubicin decrease intracellular activation	avoid
Radiation	increased toxicity	radiation sensitizer	monitor
Paclitaxel followed by doxorubicin	increased neutropenia and stomatitis	reduced doxorubicin clearance	use paclitaxel after doxorubicin
Dactinomycin	increased radiation recall pneumonitis	additive effects	caution
Phenytoin	reduced phenytoin levels	unknown	caution, check levels
Cyclosporin	increased hematologic toxicity	reduced doxorubicin clearance/ metabolism	caution
Calcium channel blockers	increase cardiotoxicity	additive effects	avoid
High dose progesterone	increased hematologic toxicity	unknown	caution
Cucurmin (Turmeric)	may reduce effect of Doxorubicin	inhibits Doxorubicin induced apoptosis	avoid concomitant use
Vincristine	seizures	unknown	caution

I RECOMMENDED CLINICAL MONITORING

Recommended Clinical Monitoring	Suggested Clinical Monitoring
• Clinical exam for symptoms of CHF • Periodic cardiac tests for all patients with cardiac risk factors or patients at or above the threshold dose levels (400mg/m^2 for 21 day schedules and 550mg/m^2 for weekly schedules) • Baseline and regular liver function tests	• Baseline cardiac function tests (Echo, RNA and/or MUGA scans) for all patients with cardiac risk factors

J REFERENCES

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Somasundaram S, Edmund NA, Moore DT, Small GW, Shi YY, Orlowski RZ. Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer. Cancer Res 2002 Jul 1;62(13):3868-75.

Von Hoff DD, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 1979;91:710-7.

A

DRUG NAME: PACLITAXEL

SYNONYM(S): NSC-125973

COMMON TRADE NAME(S): Taxol® (Bristol-Myers Squibb)

B

MECHANISM OF ACTION AND PHARMACOKINETICS

At the turn of the century, a British official in India noticed that extracts of the European yew (*Taxus baccata*) were being used in a clarified butter preparation for treating cancer. However, it was not until 1962 that extracts from the bark of the Pacific yew (*Taxus brevifolia*) were supplied to the National Cancer Institute by the U.S. Forest Service. An extract, paclitaxel, was randomly chosen for testing for cytotoxic activity as part of a large-scale screening program and demonstrated activity against murine tumour lines. Paclitaxel's structure was determined in 1971 and its mechanism of action in 1979. Phase I trials started in 1983, but were hampered by the frequency and severity of hypersensitivity reactions. Phase II trials began in 1988. Unlike other antimicrotubule agents in clinical use (e.g., vincristine, colchicine) that inhibit mitotic spindle formation, paclitaxel promotes assembly of microtubules, stabilizes them against depolymerization and inhibits cell replication. Taxol® is obtained via a semisynthetic process from *Taxus baccata*.

Oral Absorption	Not absorbed orally	
Distribution	Extensive extravascular distribution and/or tissue binding. Pharmacokinetics are non-linear with a disproportionate increase in AUC and C_{max} with increasing dose.	
	Cross blood brain barrier?	No
	PPB	89%
Metabolism	Hepatic metabolism (cyp 2C8 and cyp 3A4) and biliary secretion probably account for the majority of elimination	
	Active metabolite(s)	No information found
	Inactive metabolite(s)	Hydroxylated metabolites
Excretion	High concentrations found in bile; 71% excreted in feces (5% unchanged)	
	Urine	2-13% as unchanged drug
	t ½	9.9 hours (3 hr infusion)

C

INDICATIONS AND STATUS

- * Breast cancer (adjuvant, second line)
- * Non-small cell lung cancer (first line)
- * Ovarian cancer (first line combination, second line)
- * Aids related Kaposi's sarcoma (refractory to liposomal anthracycline)

Other uses include:
Head and neck cancer
Prostate Cancer

* ***Health Canada approved indication***

D

ADVERSE EFFECTS

ORGAN SITE	SIDE EFFECT	ONSET
Cardiovascular	Bradycardia (3%, transient) Severe conduction abnormality (<1%)	I
	Edema 21%, Cardiac Failure (rare)	E
	Syncope (rare)	
	Hypotension during infusion (12%)	I
	Pulmonary Embolus (rare)	
	Acute myocardial infarction (rare)	E
	Abnormal ECG (13-23%)	E
Dermatologic	Alopecia (87-93%)	E
	Rash (rare, may be severe)	
	Nail changes (2%)	E D
	Radiation recall reaction (rare)	I
Extravasation hazard (refer to **Appendix 2**)	**IRRITANT** (cellulitis, inflammation)	I
Gastrointestinal	Nausea and vomiting (44-52%)	I
	Anorexia (25%)	E
	Diarrhea (25-38%)	I
	Pancreatitis,obstruction, perforation (rare)	
	Typhlitis (rare)	
	Mucositis (20-31%)	E

D ADVERSE EFFECTS (Continued)

ORGAN SITE	SIDE EFFECT	ONSET
Hematologic	Myelosuppression Grade 4 neutropenia 27-50% 7% grade 3 /4 thrombocytopenia	E
Hepatic	Increased liver function tests including bilirubin, transient (8-22%); hepatic necrosis (rare)	E
Hypersensitivity	Type I, anaphylactoid (41%, severe 1-2%)	I
Pulmonary	Radiation pneumonitis (rare)	
	Pneumonitis / Lung Fibrosis (rare)	E
Injection site	Phlebitis, erythema, tenderness, discomfort (4-13%)	I
Musculoskeletal	Myalgia, arthralgia (54-60%; severe 8-12%)	E
Other	Infection (20-30%)	
	Fever (12%)	
	Fatigue (17%)	
	Increased creatinine (rare)	
Neurologic	Optic neuropathy and visual disturbances	E
	Peripheral neuropathy (60-64%, severe 3-4%)	E
	Autonomic neuropathy (rare)	
	Seizures, ataxia, encephalopathy (rare)	
	Ototoxicity (rare)	

Dose-limiting side effects are underlined.
I = immediate (onset in hours to days); E = early (days to weeks);
D = delayed (weeks to months); L = late (months to years)

D ADVERSE EFFECTS (Continued)

The most frequent dose limiting events are myelosuppression, neuropathy, hypersensitivity reactions and musculoskeletal effects.

Hypersensitivity reactions typically occur in early treatment courses and within the first hour of infusion. Dyspnea, flushing, chest pain and tachycardia were the most frequent manifestations. Reactions are neither dose-related nor dependent on prior exposure to paclitaxel, and may be caused by histamine release mediated by the Cremophor EL diluent. Because of the significant risk of hypersensitivity reactions the patient must be monitored closely, a physician must be readily available, as well as emergency medications and resuscitation equipment. Anaphylaxis and severe hypersensitivity reactions (hypotension, angioedema, generalised urticaria) occur in 2% of patients and may rarely be fatal.

Myalgia and/or arthralgia tend to appear 2-8 days after taxol administration and resolve within 4-7 days. Nonsteroidal anti-inflammatory drugs are successful in relieving these symptoms.

Peripheral neuropathy may be been dose limiting and is dose related. Common symptoms include numbness, tingling and/or burning pain in a glove-and-stocking distribution. The symptoms are generally tolerable, but may be disabling especially in patients treated with doses ≥250 mg/m^2, in those treated with paclitaxel in combination with cisplatin and in those at high risk of developing neurotoxicity (e.g., prior exposure to neurotoxic agents such as cisplatin and the vinca alkaloids, diabetes mellitus, or chronic alcoholism). Below the dose of 170 mg/m^2 peripheral neuropathy is rare. Mild symptoms usually improve or resolve completely within several months after discontinuation of therapy. Pre-existing neuropathies are not a contraindication to treatment with paclitaxel. The development of severe symptoms is unusual and requires a dosage reduction of 20% for subsequent courses of paclitaxel.

Central neurotoxicity may occur and may be severe, especially in children treated at high dosage.

Paclitaxel has the potential to enhance radiation injury to tissues. While often called ***radiation recall reactions***, the timing of the radiation may be before, concurrent with or even after the administration of the paclitaxel. Recurrent injury to a previously radiated site may occur weeks to months following radiation.

Toxicity may be more severe in HIV patients, especially infection (febrile neutropenia and opportunistic infections) and neutropenia.

E DOSING

Refer to protocol by which patient is being treated. Numerous dosing schedules exist and depend on disease, response and concomitant therapy. Guidelines for dosing also include consideration of white blood cell count. Dosage may be reduced and/or delayed in patients with bone marrow depression due to cytotoxic/radiation therapy.

<u>Adults</u>:

Premedication: To minimize severe hypersensitivity reactions, patients should be premedicated with:

- dexamethasone 20 mg p.o 12 and 6 hours before (consider using 10mg for HIV patients)
- diphenhydramine 50 mg IV 30-60 minutes before, and
- cimetidine 300 mg IV or ranitidine 50 mg IV 30-60 minutes before.

In the event of a treatment delay (e.g., admixture is not available) additional doses are required.
Although not included in the product monograph, many centres have successfully modified the pre-medication regimen by giving a single 20mg IV dose of dexamethasone with diphenhydramine and ranitidine (or cimetidine) 30 minutes prior to paclitaxel infusion.

E DOSING (Continued)

Intravenous: q3w: 175 mg/m^2 over 3 hours

AIDS related KS: 135mg/m^2 over 3 hours every 3 weeks OR
100mg/m^2 over 3 hours every 2 weeks

Dosage in myelosuppression: modify according to protocol by which patient is being treated; if no guidelines available, refer to **Appendix 6** "Dosage Modification for Myelosuppression" A dose reduction of 20% is suggested for subsequent courses if grade 4 neutropenia occurs. Patients should not be retreated with paclitaxel until neutrophil and platelet counts have recovered.

Dosage with neuropathy: 20% dose reduction with grade 3 or 4 neuropathy

Dosage in renal impairment: no adjustment required

Dosage in hepatic impairment: caution and dose reduction advised in patients with moderate to severe hepatic dysfunction. Suggested are:

Bilirubin and or AST/ALT	Dose (mg/m^2)
3hr Infusion	
2-4 x ULN	135
>4 x ULN	50 or omit

Dosage after hypersensitivity:

- ***For mild symptoms*** (e.g., mild flushing, rash, pruritus) it is possible to complete the infusion under close supervision.
- ***For moderate symptoms*** (e.g., moderate rash, flushing, mild dyspnea, chest discomfort, mild hypotension),
  - Stop the paclitaxel infusion and give diphenhydramine 25-50 mg IV and methylprednisolone 125 mg IV.
  - Once symptoms have resolved, resume paclitaxel infusion at a rate of 10% of original rate for 15 minutes, then at 25% of original rate for 15 minutes, and if no further symptoms develop, continue at original rate until infusion is complete.
- ***For severe symptoms*** (e.g., one or more of: respiratory distress requiring treatment, generalized urticaria, angioedema, hypotension requiring therapy),
  - stop the paclitaxel infusion and give diphenhydramine and methylprednisolone as above. Use epinephrine or bronchodilators if indicated.
  - Do not rechallenge with paclitaxel

Dosage in the elderly: No adjustment required

Children: Safety and efficacy has not been established. Children may be at a higher risk of severe and sometimes fatal neurologic toxicity, especially with high doses, possibly related to the ethanol content of paclitaxel infusions.

F ADMINISTRATION GUIDELINES (see Appendix 3a)

- Use non-PVC equipment, including 0.22 micron in-line filter; Infuse over 3 hours.
- Prefilled in 500-1000mL bag (Normal Saline or 5% Dextrose-dilution concentration 0.3-1.2 mg/mL).
- May be infused over 1 hour-mix in 250mL bag as above (not approved by manufacturer).
- May be given as 24 hour infusion-mix in 1000mL bag and use non-PVC equipment and in-line filter; given as inpatient or using CADD pump.

G SPECIAL PRECAUTIONS

Paclitaxel is classified as dangerous goods under the Transportation of Dangerous Goods Act and must be declared as such for purpose of transportation (substance is considered flammable).

Patients should be ***pretreated*** with a corticosteroid (e.g., dexamethasone 20 mg p.o 12 and 6 hours before paclitaxel), an antihistamine (e.g., diphenhydramine 50 mg IV 30-60 minutes before) and an H_2 antagonist (e.g., cimetidine 300 mg IV or ranitidine 50 mg IV 30-60 minutes before) to ***minimize severe hypersensitivity reactions***.

Paclitaxel is **contraindicated** in patients with a history of severe hypersensitivity reactions to paclitaxel or other drugs formulated in Cremophor EL (polyethoxylated castor oil) or in patients with severe baseline neutropenia (<1500 cells/mm^3; < 1000 for patients with AIDS related Kaposi's). Severe arrhythmias may occur during infusion; patients should be appropriately managed and undergo continuous EKG monitoring during subsequent infusions.

Paclitaxel is embryotoxic and fetotoxic and reduces fertility and should not be used in ***pregnancy. Breast feeding*** is not recommended due to the potential secretion into breast milk.

Paclitaxel contains ethanol, and is administered with agents such as antihistamines which cause drowsiness. Patients should be cautioned regarding driving and the use of machinery.

H INTERACTIONS

AGENT	EFFECT	MECHANISM	MANAGEMENT
Cisplatin	increased toxicity of paclitaxel if cisplatin is given prior (within hours) to paclitaxel	decreased paclitaxel clearance	give paclitaxel <u>before</u> cisplatin
Doxorubicin (after paclitaxel with prolonged infusions)	increased neutropenia and stomatitis	higher plasma levels of doxorubicin and doxorubicinol	caution

H

INTERACTIONS (Continued)

AGENT	EFFECT	MECHANISM	MANAGEMENT
Cyclophosphamide (given after paclitaxel)	increased myelosuppression	unknown	caution
Radiation	radiation pneumonitis	increased pulmonary effects	avoid/caution
Carboplatin (given after paclitaxel)	reduced thrombocytopenia	unknown	caution
Substrates, inducers or inhibitors of CYP2C8 and/or CYP3A4 (St john's wort, ketoconazole, Verapamil, etoposide, decadron, protease inhibitors)	increased toxicity or decreased effect	alteration of pharmacokinetics of paclitaxel	use caution

I

RECOMMENDED CLINICAL MONITORING

Recommended Clinical Monitoring

- Clinical assessment of fever, infection, neurologic (Sensory), hypersensitivity and flu-like symptoms toxicity ratings at each visit
- Blood pressure and pulse rate monitoring during infusion, cardiac monitoring with prior arrhythmia
- Baseline and regular CBC
- Baseline and regular liver function tests

Suggested Clinical Monitoring

- Baseline and regular renal function tests (AIDS related Kaposi)

J

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Cancer Drug Manual (the Manual), 1994, British Columbia Cancer Agency (BCCA)

Compendium of Pharmaceuticals and Specialities. 2006. Taxol®. Canadian Pharmacists Association.

ANTICANCER RESEARCH 8: 355-368 (1988)

Correlation of *in Vitro* Cytotoxicity with Preclinical *in Vivo* Antitumor Activity

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Abstract. *Several human and murine tumor cell lines were evaluated in an* in vitro *cytotoxicity assay as prescreens for fermentation extracts and pure materials subsequently tested in* vivo *against P388 leukemia or B16 melanoma. Each material, regardless of its* in vitro *cytotoxicity, was evaluated* in vivo. *At the criteria levels of* in vitro *positivity and* in vivo *activity invoked, a highly significant relationship between these two endpoints was demonstrated for each cell line. When cell lines were compared, most of them performed in a similar manner, with HCT-116 human colon carcinoma cells providing a modest advantage predicting for P388 activity in some comparisons. Using the data from any two cell lines in concert did not improve the acuity of the prescreen beyond that associated with the better cell lines used singularly and only a minority of active materials was predicted for uniquely. Overall, the* in vitro *cytotoxicity assay provided a useful prescreen for selecting P388 and B16* in vivo *active materials.*

Faced with the prospect of having to evaluate thousands of materials for their potential *in vivo* antitumor activity, one would prefer to reduce the sample size through the use of an efficient *in vitro* prescreen. Such a prescreen should be adequately sensitive to the materials which would yield active results *in vivo*, but also discriminating enough to accurately predict both active and inactive materials.

Although the emphasis, as of late, is to screen potential antitumor materials for their effect on various histological types of human tumor cell lines, to be followed, ideally, by confirmation of activity and selectivity in an *in vivo* human tumor xenograft model (1, 2), the established method of selecting potential clinical drug candidates on the basis of their activity against murine tumor models remains a valid approach to cancer drug development which deserves continued refinement (3-5). We undertook a survey of several human and murine tumor cell lines and evaluated their usefulness as in vitro prescreens for materials subsequently tested in one or more *in vivo* murine tumor models. The materials evaluated included relatively pure compounds as well as mixtures of unknown substances in fermantation extracts. Not only did we test *in vivo* those materials which were found to be sufficiently cytotoxic in the prescreen assays, but we tested everything *in vivo* including the prescreen-negative samples. This allowed us to judge the sensitivity and true negative rates (TNR) of each cell line used as a prescreen, rather than only the true positive rates (TPR).

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Key Works: Cytotocixity, antitumor, preclinical.

Materials and Methods

Compounds and fermentation extracts. All materials tested were either synthesized or fermented at the Pharmaceutical Research and Development Division of Bristol-Myers Co., Inc., or submitted as potential licensing agents to the same organization. Compounds were either dissolved in an aqueous-based vehicle, initially dissolved in dimethyl sulfoxide (DMSO) followed by dilution in an aqueous-based vehicle, or (for in vivo studies) suspended in an aqueous-based vehicle with Tween 80 or carboxymethylcellulose as a suspending agent. Fermentation broths were diluted with water; solids extracted from fermentation broths were either diluted with water or initially dissolved in DMSO followed by dilution with water.

With respect to the fermentation samples, they represented a rather select group in that the cultures (organisms) from which they were derived had been initially screened and found to produce a positive effect in Bristol-Myers Research Institute in Tokyo, Japan. The screening tests conducted in Japan included both eukaryotic cell cytotoxicity assays very similar to those described herein, as well as various prokaryotic assays (6, 7). Cultures yielding positive results in Japan did not always confirm their activity in our laboratories, nor were all fractions of a given culture necessarily positive in cytotoxicity assays. Nevertheless, it is important to note that our fermentation samples were not randomly obtained from nature; they represent a population enriched for its potential cytotoxicity. How these selected cultures may differ from randomly selected cultures (with regard to the evaluations conducted herein) is not known, but it was pragmatic for us to use such an enriched population in our investigations. There are no *a priori* reasons that the use of cultures enriched for cytotoxic activity would necessarily compromise the applicability of our findings to the situations in other settings.

For *in vitro* cytotoxicity assays, the highest concentration of DMSO used when evaluating a material was 1.5%, a concentration found to have no appreciable cytotoxic effect against the cell lines employed. For *in vivo*

antitumor assays, the highest concentration of DMSO used was 10% of the injected volume.

Cell lines. Established tumor cell lines of both murine and human origin were used for *in vitro* cytotoxicity assays. The cell lines used of murine origin included: B16-F10 melanoma; M109 lung carcinoma; and C26 colon carcinoma. The cell lines used of human origin included: HCT-116 colon carcinoma; RCA colon carcinoma; Moser colon carcinoma; and KB nasopharengyl carcinoma.

Animals. Inbred DBA/2, C57BL/6, and hybrid CDF_1 (Balb/c × DBA/2)F_1 and BDF_1 (C5BL/6 × DBA/2)F_1 mice of both sexes, 16 to 20 g, were used for *in vivo* antitumor testing. All mice were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA).

Tumors. P388 leukemia was maintained in ascitic form in DBA/2 mice. B16 melanoma was maintained as a sc growing tumor in C57BL/6 mice.

In vitro cytotoxicity assay. The methology for the anchorage-dependent cell cytotoxicity microtiter assay using a colorimetric endpoint has been published previously (8,9). Briefly, 4 × 10^3 monodispersed cells were added to each well of a 96-well microtiter plate and incubated overnight at 37°C in 5% CO_2 in air to permit cell attachment. The cells were then exposed to four-fold serial dilutions of the test materials and incubated for an additional 72 hr. All assays were performed in duplicate. At the end of the incubation period, detached cells and media were removed, the remaining cells were fixed, stained (with crystal violet), washed and optical densities determined using a Dynatech MR600 plate reader at 590nm. The concentration of each material required to inhibit cell growth by 50% of control values (IC50) was determined for each cell line assayed using a linear regression analysis of the absorption data. The correlation data described in the Tables reflect the proportions of materials whose IC50 values meet or exceed the various endpoint criteria listed.

P388 cells were not used in our *in vitro* cytotoxicity assay; their growth in suspension cultures and the unavailability (when we began this study) of efficient vital stain dye technology was inconsistent with mass production prescreening.

In vivo antitumor assays. Experiments involving P388 were initiated by implanting 10^6 leukemic cells ip into CDF_1 mice. Each compound or fermentation broth-treated group consisted of 4 (fermentation materials) or 6 (pure compounds) mice, and leukemia control groups were composed of 10 mice. B16 experiments were begun by implanting 0.5 ml of a 10% tumor brei suspension, ip, or 25 mg (approximately) tumor fragments sc (via trochar), into BDF_1 mice. Both compound- and fermentation broth-treated, and control groups, consisted of 10 mice.

P388 experiments were terminated on Day 30 and B16 experiments were terminated on Day 60. Mice alive at the end of an experiment were autopsied and judged to be cured if no signs of disease were visible. Each material in an experiment was evaluated at a minimum of 3 dose levels for each treatment schedule used. The treatment schedules used in P388 experiments were either single injections on Day 1 or five consecutive daily injections beginning on Day 1. For B16 experiments, the treatment schedules used were either nine consecutive daily injections beginning on Day 1, or intermittent injections given on Days 1,5 and 9. Treatment of mice bearing ip P388 and B16 was always by the ip route whereas mice bearing sc B16 were generally treated ip with the test material unless it was sutuble, in which case intermittent iv treatments were often used.

Mice were observed daily, and antitumor activity of each tested material was determined based upon the median extension of lifespan in treated mice (T) compared to control mice (C) expressed as % T/C (10). A material was considered active if it produced a T/C ≥125% versus ip P388 or B16, and a T/C ≥135% versus sc B16. Material-treated mice dying prior to Day 5 in P388 experiments, or Day 10 in B16 experiments, were presumed to have died from treatment-associated toxicity and were excluded from calcuations of % T/C. No result of therapy was used in which deaths attributable to material toxicity exceeded 17% in the treated group.

Definition of terms. In all of the definitions used, "true" and "false" refer to the outcome in the *in vivo* tumor models. The terms "active" and "inactive" are reserved for *in vivo* results whereas "positive" and "negative" describe *in vitro* outcomes. Thus, a true positive (TP) is a material whose *in vivo* activity was accurately predicted for *in vitro* by virtue of having had an IC50 value which satisfied the declared criteron for positivity. In this manner, the definitions of true negative (TN), false positive (FP) and false negative (FN) can be logically derived. TPR is defined as the No. of TP responses divided by the sum of the No. of TPs plus No. of FPs. TNR is equal to the No. of TNs divided by the sum of the No. of TNs plus No. of FNs. Sensitivity (SENS), which is a measure of how well the prescreen detects all the in vivo active materials assayed, is defined as the No. of TPs divided by the sum of the No. of TPs plus No. of FNs. Yield, which is a measure of the proportion of materials assayed *in vitro* that pass a given criterion for prescreen positivity, is defined as the sum of the No. of TPs plus No. of FPs divided by the total No. of samples assayed. The prevalence (PREV) of a sample population is the percentage of *in vivo* actives contained in the sample. Note that the yield of an *in vitro* assay can be determined without performing an *in vivo* test and, conversely, that PREV has no relationship with an *in vitro* assay.

Correlating in vitro *and* in vivo *assay results.* For all materials tested, comparisons were made of their IC50 values (in either µg/ml or dilution from initial sample) versus each of several cell lines and the maximum % T/C value obtained in whatever tumor models were investigated. Correlations were determined using IC50 criteria for positive *in vitro* effects which were varied over several concentrations or dilutions. For each *in vitro* IC50 criterion used, the number of TP, FP, TN, and FN outcomes was determined. From these parameters we derived TPR, TNR, sensitivity and yield values which, in relation to the underlying prevalences, were used to compare the predictive worth of each cell line as a prescreen for the various *in vivo* assays.

Statistical evaluations. Standard contingency table procedures were used to obtain TPR, TNR, sensitivity and yield (11). McNemar's test (11, 12) was used to compare the equivalences of *in vivo* and *in vitro* proportions. The significance of each cell line's relationship between *in vitro* positivity and *in vivo* activity was then analyzed by a follow-up Chi-square test to McNemar's test (12). Although these results are not reported, there was a highly significant ($p < 0.0001$) relationship for ip P388 and ip B16 data for each cell line.

Statistical comparison of rates between tumor lines was made by determining the sample sizes needed to detect a range of differences in proportions from 5 to 10% given a significance level of 0.05 and a power of 0.80 (11). Those comparisons with sufficient sample size for the difference found in the data to be significant were so described. This method is mathematically equivalent to the usual two-sample test of equality of two binomial proportions.

Results

Correlation of in vitro cytotoxicity data with in vivo P388 antitumor data. The in vitro cytotoxicity data from seven tumor cell lines exposed to fermentation samples were correlated with the antitumor data from *in vivo* P388 leukemia experiments involving the same samples. Activity *in vivo* was defined as a T/C ≥ 125% and the criteria for positivity *in vitro* were varied between IC50 dilutions of 1-256, 1-512 and 1-1,024. These correlations are summarized in Table I.

Upon perusal of these data one realizes that the various correlation values associated with each cell line do not differ appreciably from one another. For example, regardless of the dilution level chosen for a positive effect *in vitro* (between

1-256 and 1-1,024), the TPRs for all the cell lines varied only between 40 to 53%. With only one exception (KB cells at 1-1,024 IC50 dilution), TPR values increased gradually with increasing stringency of the *in vitro* positivity criteria (and decreasing yield). The underlying prevalences associated with the sample populations subjected to P388 testing varied from 28 to 33%. Thus, each cell line yielded TPRs in excess of the prevalence associated with the subset of samples evaluated. Additionally, each cell line's TPRs exceeded 1-TNR, thereby indicating the relative worthiness of a positive prediction (see Discussion).

With respect to the other correlation parameters presented in Table I, namely TNR and sensitivity, their values decreased with increasing stringency of criteria for positive *in vitro* effects. TNRs varied only slightly more than TPRs over the range of IC50 levels inspected, 71% to 91% when considering all seven cell lines collectively. For any single cell line, however, TNRs varied by only 13 percentage points or less. The parameter which varied the most as the dilution levels for positivity were changed was sensitivity. A low sensitivity value of 29% was obtained with C26 at a 1-1,024 dilution compared to a 90% sensitivity value obtained with HCT-116 at a 1-256 dilution. For some cell lines such as KB, C26 and B16-F10, the sensitivity value observed at the 1-1,024 dilution represented a decrease of more than 50 percentage points compared to the sensitivity value observed at the 1-256 dilution. Other cell lines, such as HCT-116 and RCA, had sensitivity values which were much less influenced by changing the IC50 criterion for positivity between 1-256 and 1-1,024.

As shown in Figures 1A and 1B, TPR and, particularly, sensitivity were quite dependent on the yield of all the cell lines whose cytotoxicity data were used to detect and predict fermentation samples having in vivo P388 activity. In order to compare cell lines with respect to their relative TPR, TNR, and sensitivity, an IC50 was chosen for each cell line such that their yields would be comparable. The yields associated with the IC50 criteria selected ranged from 32 to 37%, and except for the HCT-116 and RCA cell lines whose selected IC50 was a 1-1,024 dilution, the IC50 of all the other cell lines consistent with these yields was a 1-512 dilution (Table II).

The TPR, TNR, and sensitivity associated with HCT-116 cells was greater than for any of the other cell lines evaluated and in several instances the differences were statistically significant ($p < 0.05$). For many comparisons however, between HCT-116 and other cell lines, the advantages shown by the former were not statistically significant, in some instances due to insufficient sample sizes.

Would the correlation values determined for individual cell lines improve if one considered the collective data derived from two cell lines? This question was addressed by combining the data from each pairwise cell line comparison posible but, for the sake of convenience, only using a single IC50 criterion for positivity *in vitro* of 1-512 dilution. In the particular analysis to be discussed, a positive in vitro result was defined as an IC50 achieved by *either* cell line in each pairwise comparison. The results of the analysis are shown in Table III.

Included in Table III are the correlation values for several parameters determined for individual cell lines. Note that these values differ slightly from the equivalent determinations presented in the previous Tables, due to the slightly larger sample sizes amassed at the time of the analysis. Additionally, in performing the pairwise evaluations, only data from samples tested against each cell line in a given combination were used. With the exception of one pairwise analysis (HCT-116 with C26 cells), no cell line combination yielded TPR or TNR values significantly greater than the more effective cell line in each pair. A strict comparison of correlation values between cell line pairs versus individual cell lines would involve deriving those values for the relevant sample subsets. Each cell line would have six sets of calculations required to be used in conjunction with each possible cell line pair. For reasons of practicality, only the correlation values associated with the total historical experience gained with each cell line was included in Table III (data from individual cell lines were, however, analyzed with respect to TPRs for sample subsets associated with each cell line pair and these values can be found in Table IV).

With respect to the cell line pair HCT-116 and C26, which had a TPR of 57% based upon a positive result against either cell being construed as a positive *in vitro* result, the sample size of 266 represents only a fraction (22%) of HCT-116's total sample size. Accordingly, the apparent advantage, with respect to relative TPRs, of this cell line pair versus its individual constituents (57% vs ≤ 49%) should be reassessed using individual HCT-116 and C26 TPRs associated with the same 266 materials. These values are presented in Table IV and were 61% and 59% for HCT-116 and C26 cells, respectively. Thus, the 57% TPR for this cell line pair (using the principle described for analysis in Table III) was not greater than the relevant TPRs for each individual cell line.

Whereas TPRs and TNRs for paired cell lines were not significantly improved compared to the total historical data for individual cell lines (Table III), sensitivities associated with selected cell line combinations were found to increase. For example, the sensitivity associated with B16-F10 cells plus M109 cells was 60%, whereas the individual cell line sensitivities for this pair were ≤ 48%. The increased sensitivity may be due in large part to the improved yield of the cell line pair (39%) compared to the individual cell line yields (32-33%), but interpolating from Figure 1A one would have anticipated only a 56% sensitivity. Additional cell line pairs also were found with improved sensitivities compared to their constituent members (KB plus Moser, KB plus B16-F10, C26 plus M109, etc.).

The results just described evolved as a consequence of merging individual cell line data and analyzing it on the basis of *either* cell line in a pair having yielded a positive *in vitro* effect being construed as a positive effect for the pair. In the section to follow, comparisons of pooled data for cell line pairs were once again performed but the analysis was conducted under a

Table I. *Evaluation of* in vitro *cytotoxicity data and* in vivo *P388 leukemia data for fermentation materials: Effect of varying positivity criteria*[a].

Cell line	*In vitro* dilution	No. of comparisons	Parameters calculated				
			Yield	PREV	TPR	TNR	SENS
KB	1-256	1154	.54	.33	.47	.84	.77
	1-512	»	.36	.33	.48	.75	.51
	1-1,024	»	.25	.33	.44	.71	.34
HCT-116	1-256	1155	.66	.31	.42	.91	.90
	1-512	»	.49	.31	.47	.84	.74
	1-1,024	»	.37	.31	.52	.81	.61
Moser	1-256	1357	.54	.30	.42	.85	.77
	1-512	»	.37	.30	.44	.78	.54
	1-1,024	»	.26	.30	.51	.78	.45
RCA	1-256	799	.63	.31	.44	.89	.87
	1-512	»	.46	.31	.47	.82	.69
	1-1,024	»	.37	.31	.47	.78	.56
C26	1-256	419	.57	.32	.41	.81	.74
	1-512	»	.35	.32	.49	.78	.54
	1-1,024	»	.17	.32	.53	.73	.29
B16-F10	1-256	1311	.50	.28	.40	.83	.71
	1-512	»	.32	.28	.40	.77	.45
	1-1,024	»	.20	.28	.44	.76	.32
M109	1-256	972	.51	.30	.42	.83	.72
	1-512	»	.33	.30	.44	.77	.48
	1-1,024	»	.24	.30	.46	.76	.38

[a]Activity *in vivo* versus P388 was based on a T/C of ≥125%.

different rule: a positive *in vitro* effect for a cell line pair was interpreted to have occured only if *both* cell lines satisfied the positivity criterion of an IC50 of ≥ 1-512 dilution.

The majority of TPs, as well as FPs, were detected in common by both cell lines in every pair. The percentage of «common TPs» as a function of all TPs ranged from 51% (Moser plus C26 cells) to 96% (HCT-116 plus RCA cells). Consequently, the incidence of TPs unique to any one cell line was always less than half of all TPs, and, in fact, never exceeded 43% (C26 cells when paired with Moser cells). Nevertheless, a few cell lines, e.g. HCT-116 and RCA, had ≥ 23% of all TPs attributable to them uniquely in the majority of pairwise situations. Occasional cell line pairs, e.g. KB plus Moser or B16-F10 cells, exhibited a high degree of discordance between their constituent members, with respect to the detection and prediction (TPs) of P388 active materials, thus making these pairs potentially useful due to their complementary relationship.

The presence of TPs must always be tempered by the incidence of FPs which together contribute to the TPR. Included in Table IV are the TPRs for each cell line pair when only the in vitro positive results obtained in common were analyzed («common TPR»), as well as TPRs for each member of each cell line pair regardless of what the other cell line predicted, and finally the TPRs of each member of each cell line pair when only one cell line yielded a positive in vitro result («unique TPR»). Depending upon the subpopulation of samples contained in a given analysis, the TPRs associated with each cell line in the six possible pairwise combinations were found to vary considerably. For example, the TPR for KB cells when analyzed using the 965 samples contained in the KB plus M109 cell pair was 38%, but the TPR for KB cells among the 1,083 samples subjected to the KB plus Moser cell pair was 46%. Other examples of >5% variation in TPRs for a given cell line were observed, especially for the smaller sample sizes associated with the cell line pairs containing C26 cells.

With respect to the «common TPR» values generated for each cell line pair, these varied from 41 to 66% (the higher values were all associated with small sample sizes involving cell line pairs containing C26 cells), but generally ranged from 41 to 51%. There was no instance in which a common TPR significantly exceeded the greater of the two TPRs associated with the individual cell lines in a two-line combination. There were several occasions where the common TPR was inferior to the individual TPR of one of the constituent cell lines in a pair, but never to a statistically significant extent given the sample sizes.

With respect to «unique TPRs», there were definitely in-

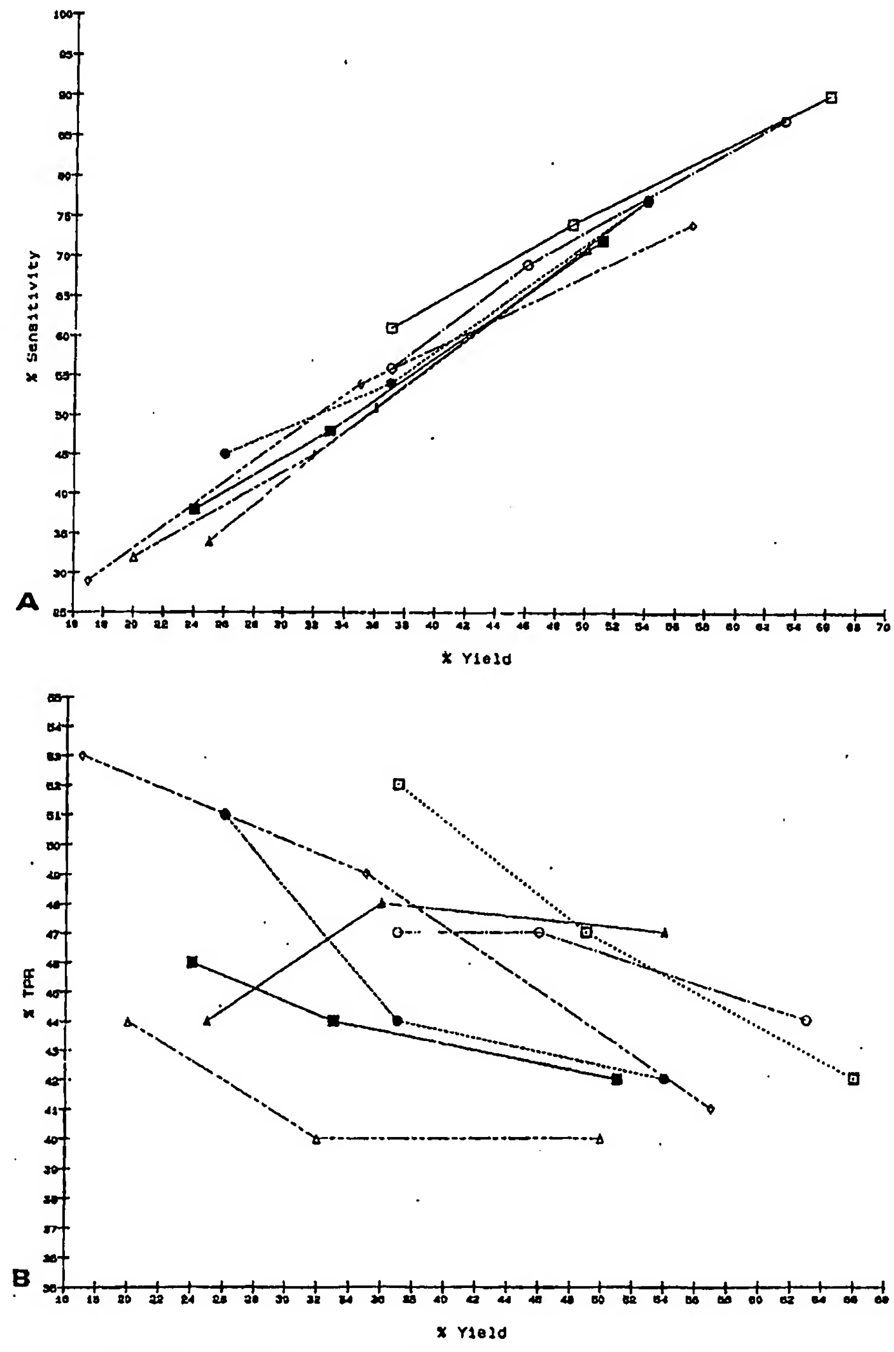

Figure 1, A and B. *Relationship of yield to sensitivity (A) or true positive rate (TPR) (B) for* in vitro *cytotoxicity versus* in vivo *P388 activity of fermentation samples using the following cell lines* in vitro: *KB (▲); Moser (•); RCA (○); C26 (◊); B16-F10 (△); M109 (■); and HCT-116 (□).*

Table II. *Selected cell line cytotoxicity data versus P388* in vivo *data for fermentation materials*[a].

Cell line	n	IC50 dilution	Parameters calculated			
			Yield	TPR	TNR	SENS
KB	1154	1-512	.36	.48	.75[b]	.51[b]
HCT-116	1155	1-1,024	.37	.52	.81	.61
Moser	1357	1-512	.37	.44[b]	.78	.54[b]
RCA	799	1-1,024	.37	.47	.78	.56
C26	419	1-512	.35	.49	.78	.54
B16-F10	1311	1-512	.32	.40[b]	.77	.45[b]
M109	972	1-512	.33	.44[b]	.77	.48[b]

[a]The % Yield (# TP + # FP/Total n size) was similar (as shown) for each cell line at the IC50 dilutions chosen, and the underlying proportion of *in vivo* active samples ("prevalence") was also very similar (29-33%).
[b]$p<0.05$ between this value and HCT-116's value.

stances in which one cell line had a better such TPR than the other cell line in a pairwise combination. For example, HCT-116 yielded unique TPRs which always exceeded the comparable TPRs associated with each cell line matched with it. In certain other pairwise comparisons, e.g. Moser with RCA, C26 or B16-F10 cells, the unique TPRs of each constituent were very similar. Thus, while only RCA cells detected 27% of all the TPs in the pair composed of Moser plus RCA, compared to only 4% of all the TPs described uniquely by Moser cells, both cell lines had identical unique TPRs due to the same proportion of unique FPs to TPs associated with them.

The *in vitro* cytotoxicity data from seven tumor cell lines exposed to chemically-defined compounds were correlated with the antitumor data from *in vivo* P388 leukemia experiments involving the same samples. Activity *in vivo* was defined as a T/C of ≥125% (just as was done for the fermentation samples) and the criteria for positivity *in vitro* were varied between IC50 values of 10, 25, and 100 μg/ml. These correlations are summarized in Table V.

Two of the cell lines, HCT-116 and RCA, were used less frequently than the other five cell lines and consequently the number of samples tested *in vitro* against them was reduced. The smaller sample sized associated with these two cell lines also had prevalences (56%) which differed from the prevalences associated with the other cell lines (45-48%). Because of these differences (in sample size and prevalence) data involving HCT-116 and RCA were included in Table V but were considered separately in the discussion of the correlation data.

Yields associated with each cell line used for in vitro screening decreased with increasing stringency of the IC50 criteria required for a positive effect. For yields of approximately 33% (32-35%), it would be necessary to invoke IC50s of 10 or 25 μg/ml, depending upon the cell line. HCT-116 had a yield of 42% at the lowest IC50 value (10 μg/ml) used for analysis, and so this cell line probably would have required using an IC50 of 5 μg/ml in order to reduce its associated yield to 33%. At yields approximating 33% the sensitivities associated with the five predominantly used cell lines ranged from 58% to 65%, not a significant difference given the sample sizes involved. Should one desire higher sensitivities, or have in vivo testing capacity which can tolerate a greater yield, then a less stringent positivity criterion (higher IC50 value) could be invoked.

The five cell lines, when considered at IC50s associated with nearly identical yield values, also had very similar TPRs. This parameter ranged from 83 to 89%, and thus was much higher than the TPRs found while testing fermentation samples against the same cell lines at quite similar yield values (cf Table II and Table V).

TNR values were the least affected by changing cell lines. At the IC50s which gave similar yields among the five predominantly used cell lines, the TNRs for those lines ranged from 70 to 74%. Thus, among the five cell lines, KB, Moser, C26, B16-F10 and M109, when considered at IC50s associated with similar yields, very small differences were observed with respect to sensitivity, TPR and TNR.

Regarding RCA and HCT-116, the most notable facet of their data evaluation was the finding of TPRs in excess of 90%. RCA, when used with an IC50 of 10 μg/ml, had a yield of 32% and a TPR of 98%. This TPR was achieved with a sensitivity of 56%, which was not greatly reduced from the 58 to 65% sensitivity values observed with the aforementioned five cell lines at similar yields, and a TNR of 64%, also not much lower than the 70-74% TNRs seen with the other cell lines.

HCT-116 was not evaluated at IC50 values low enough to give less than a 42% yield. Thus, the 93% TPR found at the 42% yield level could ostensibly be improved upon if a more stringent IC50 value was utilized. Conceivably, both the TNR and sensitivity values would decrease at a slightly lower IC50 criterion, but the potential for HCT-116 to achieve an even better TPR (than 93%) and yet retain good sensitivity and TNR values is considerable. If one inspects the correlation parameters for each cell line at IC50 values which gave yields approximating 42%, HCT-116 can be seen to fair quite well in comparison to the other cell lines, particularly with respect to comparative TPRs.

Correlation of in vitro cytotoxicity data with in vivo B16 antitumor data. The *in vitro* cytotoxicity data from three cell lines (HCT-116, Moser and B16-F10) exposed to fermentation samples were correlated with the antitumor data from *in vivo*

Table III. *Correlations of* in vitro *cytotoxicity data and* in vivo *P388 data for fermentation materials using single and multiple cell line analyses (assessed at an IC50 of 1-512 dilution)*[a].

Cell line(s)	Incidences					Parameters calculated		
	n	TN	FP	FN	TP	TPR	TNR	SENS
KB	1163	561	221	185	196	.47	.75	.51
HCT-116	1203	508	318	94	283	.47	.84	.75
Moser	1405	683	297	187	238	.44	.79	.56
RCA	808	353	205	78	172	.46	.82	.69
C26	419	211	75	61	72	.49	.78	.54
B16-F10	1359	709	261	212	177	.40	.77	.46
M109	981	509	184	149	139	.43	.77	.48
KB & HCT[b]	901	362	235	80	224	.49	.82	.74
KB & Moser	1083	492	243	116	232	.49	.81	.67
KB & RCA	803	332	221	73	177	.45	.82	.71
KB & C26	415	188	94	44	89	.49	.82	.67
KB & B16[c]	996	456	234	114	192	.45	.80	.63
KB & M109	965	441	238	122	164	.41	.78	.57
HCT & Moser	1154	464	330	81	279	.46	.85	.78
HCT & RCA	668	253	194	54	167	.46	.82	.76
HCT & C26	266	114	54	27	71	.57	.81	.72
HCT & B16	1150	479	326	80	265	.45	.86	.77
HCT & M109	830	352	221	69	188	.46	.84	.73
Moser & RCA	788	341	211	65	171	.45	.84	.73
Moser & C26	397	193	77	50	77	.50	.79	.61
Moser & B16	1295	582	336	127	250	.43	.82	.66
Moser & M109	966	454	227	106	179	.44	.81	.63
RCA & C26	171	84	38	17	32	.46	.83	.65
RCA & B16	753	321	215	57	160	.43	.85	.74
RCA & M109	749	318	213	59	159	.43	.84	.73
C26 & B16	367	179	75	50	63	.46	.78	.56
C26 & M109	347	168	81	37	61	.43	.82	.62
B16 & M109	952	461	205	115	171	.46	.80	.60

[a]A positive *in vitro* result when using more than one cell line was construed for the purpose of this analysis as an IC50 obtained with *either* cell line which met the specified IC50.
[b]HCT-116 cells
[c]B16-F10 cells

B16 experiments involving the same samples. *In vivo* experimentation consisted of either ip or sc tumor implantations. The correlation between *in vitro* cytotoxicity data and *in vivo* ip B16 data will be discussed first (Table VI).

The sample sizes available for evaluation were modest compared to those contained in the P388 data sets, ranging from 125 to 147. Three different criteria for *in vitro* positivity were evaluated: IC50s of 1-256, 1-1,024 and 1-4,096 dilutions. Correlations between *in vitro* cytotoxicity and *in vivo* activity versus ip B16 were made at each of these criteria of positivity. The yields at each IC50 level evaluated were similar for B16-F10 and Moser cells but HCT-116 cells were more sensitive. In order to eliminate from contention at least one-half the sample population tested (i.e. have the yield fall below 50%), the positivity criterion for B16-F10 and Moser cells had to be established at an IC50 of 1-1,024 dilution whereas the IC50 criterion for HCT-116 cells was set at 1-4,096 dilution. The correlation values determined for these cell lines at these IC50 values are shown in Table VI.

The underlying prevalences for the sample populations tested against the three cell lines were comparable (37-39%). Additionally, the TPRs for each cell line were very similar (50-53%). The TNRs and sensitivities varied slightly between cell lines but not significantly; nevertheless, Moser cells led both of these correlation categories whereas HCT-116 and B16-F10 cells were comparable to one another.

Would the predictive ability of in vitro cytotoxicity data improve if the results from more than one cell line were considered simultaneously? This type of analysis was performed in two ways. The first method involved defining positivity *in vitro* as achieving a specified IC50 for either (or any) cell line in a two (or three) cell lines analysis. For data analyzed in this

Table IV. *Extent of accordance and discordance among cell lines with respect to correlations between* in vitro *cytotoxicity and* in vivo *P388 leukemia data for fermentation materials (assessed at an IC50 of 1-512 dilution).*

Cell lines		n	Incidence (% of total category)						% TPR:				
			True positives			False positives			A&			A+	B+
A	B		Common	Unique A	Unique B	Common	Unique A	Unique B	B	A	B	B−	A−
KB	HCT[a]	901	146(65)	5(2)	73(33)	157(67)	28(12)	50(21)	48	45	51	15	59
KB	Moser	1083	133(57)	52(22)	47(20)	155(64)	63(26)	25(10)	46	46	50	45	65
KB	RCA	803	107(60)	5(3)	65(37)	155(70)	18(8)	48(22)	41	39	46	21	58
KB	C26	415	62(70)	17(19)	10(11)	50(53)	21(22)	23(24)	55	53	50	45	30
KB	B16[b]	996	105(55)	41(21)	46(24)	135(58)	74(32)	25(11)	44	41	49	36	65
KB	M109	965	106(65)	26(16)	32(20)	155(65)	58(24)	25(11)	41	38	43	31	56
HCT	Moser	1154	192(69)	81(29)	6(2)	249(75)	67(20)	14(4)	44	46	43	55	30
HCT	RCA	668	144(86)	14(8)	9(5)	138(71)	25(13)	31(16)	51	49	48	36	23
HCT	C26	266	54(76)	16(23)	1(1)	28(52)	16(30)	10(19)	66	61	59	50	9
HCT	B16	1150	162(61)	102(38)	1(0)	213(65)	101(31)	12(4)	43	46	42	50	8
HCT	M109	830	120(64)	66(35)	2(0)	141(64)	67(30)	13(6)	46	47	44	50	13
Moser	RCA	788	118(69)	6(4)	47(27)	149(71)	7(3)	55(26)	44	44	45	46	46
Moser	C26	397	39(51)	5(6)	33(43)	33(45)	5(6)	37(48)	53	52	50	50	47
Moser	B16	1295	140(56)	74(30)	36(14)	212(63)	81(24)	43(13)	40	42	41	48	46
Moser	M109	:966	111(62)	40(22)	28(16)	139(61)	43(19)	45(20)	44	45	43	48	38
RCA	C26	171	27(84)	5(16)	0(0)	30(79)	3(8)	5(13)	47	49	44	63	0
RCA	B16	753	107(67)	45(28)	8(5)	126(59)	78(36)	11(5)	46	43	46	37	42
RCA	M109	749	101(64)	53(33)	5(3)	145(68)	60(28)	8(4)	41	43	41	47	38
C26	B16	367	43(68)	17(27)	3(5)	47(63)	24(32)	4(5)	48	46	47	41	43
C26	M109	347	43(70)	11(18)	7(11)	47(58)	25(31)	9(11)	48	43	47	31	44
B16	M109	952	109(63)	34(20)	28(16)	141(69)	23(11)	41(20)	44	47	43	60	41

[a]HCT-116 cells
[b]B16-F10 cells

manner (Table VI), the positive criterion for HCT-116 cells was an IC50 of 1-4,096 dilution and for B16-F10 and Moser cells it was a 1-1,024 dilution. Although the prevalences for the sample populations evaluated remained (as expected) at the levels found for the single cell correlation data, the yields were increased noticeably, particularly for the pairs involving B16-F10 cells. For example, whereas the yields for B16-F10 and HCT-116 individually did not exceed 46%, these two cell lines when considered together had a 57% yield.

TNRs for cell line combinations were not noticeably improved relative to the higher TNR of the constituent cell lines in the various combinations. Sensitivities however, did improve marginally in the cell line combination settings compared to the values associated with the individual cell lines, but this would be expected considering the higher yields. Despite the increases in sensitivities and yields, no combination composed of two or three cell lines produced a TPR that was greater than those associated with each cell line individually.

A second method was used to analyze the possible advantage of applying in vitro cytotoxicity data from more than one cell line as a means of predicting *in vivo* B16 activity. This method differed from the previous approach in that an *in vitro* positive result for two cell lines required that both (not either) cell lines had positive IC50 values associated with the sample being evaluated. A positive *in vitro* result was defined as an IC50 dilution of 1-1,024 versus Moser and B16-F10 cells, and a 1-4,096 dilution for HCT-116 cells. Using this methodology, an analysis was performed to determine the relative predictive abilities of the various cell line combinations and the degree of accordance and discordance between indivudual cell lines with respect to their sensitivities to the sample evaluated (Table VII).

No two cell line combination yielded a TPR that was significantly greater than the better TPR associated with the individual cell lines. Stated differently, there was no advantage prescreening samples using two cell lines (rather than one) to predict for in vivo activity versus ip B16. This is shown by comparing the «A&B» % TPR column with the «A» and «B» TPR columns in Table VII. For example, considering HCT-116 and B16-F10 cell lines together, the TPR was 58%, whereas individually the TPRs were 56% and 47%, respectively. The combination TPR of 58% was not different than the 56% TPR associated with HCT-116 cells, and was not statistically different from the 47% TPR of B16-F10 cells (considering the limited sample size involved).

Part of the analysis reflected in Table VII involved a determination of the degree of uniqueness associated with each cell line's sensitivity toward the sample population evaluated. How often, for example, were HCT-116 cells sensitive to a sample (i.e. an IC50 was achieved sufficient to yield a positive *in vitro* result) but the other cell line(s) was insensitive? And how often were these unique in vitro responses accurate with respect to predicting for *in vivo* activity?

Each cell line in each pairwise combination has associated

with it unique instances of sample sensitivity. But most (68-84%) TPs were detected in common by both cell lines in the three cell line pairs evaluated and only a small minority of TPs (0-18%) were detected and predicted for by one, but not the other, cell line in each of the three cell line pairs scrutinized. Finally, no unique TPR associated with any cell line from any pairwise combination exceeded 50%, or exceeded that same cell line's TPR considered without regard to the response of the other cell line in the pair.

The sample sizes available for analysis of data from sc B16 experiments were also very limited (n sizes of 123-133). The prevalences associated with the data, as one might expect with an insensitive tumor model, were quite low (7-8%). We found that neither HCT-116, Moser nor B16-F10 cells produced a TPR, at IC50 positivity criteria of between 1-256 and 1-1,024 dilutions, which exceeded the underlying prevalences; furthermore, only rarely did we observe a TPR which was even equal to 1-TNR (data not shown).

Discussion

The application of *in vitro* assays for the purpose of prescreening samples destined for *in vivo* evaluation has long been a commonplace procedure (6,7,13-16). Recently however, with the advent of more sophisticated cell culture techniques (17-22), increased cost of hygienically-reared inbred research animals, and disenchantment with standard murine tumor models for discovering new chemotherapeutics, there is a new emphasis being placed on eukaryotic cell lines as tools for drug discovery (1,2). The adoption of new means to detect novel agents should not preclude the prudent application of traditional approaches. For the many drug discovery laboratories which intend to continue to rely on those traditional approaches, the identification and improvement of *in vitro* prescreens which correlate with *in vivo* murine antitumor activity are still needed.

For many years our own *in vitro* prescreens consisted of prokaryotic or viral DNA assays (6,7). We turned toward eukaryotic assays a few years ago and posed several questions which we would attempt to answer following the accumulation of sufficient correlative data. Based upon our findings, we planned to potentially modify our drug discovery program accordingly - an operation of some magnitude in that presently ≥ 40,000 culture fermentations are subjected annually to *in vitro* testing. The data base analyzed and presented in this report is probably unique in that hundreds of both *in vitro* prescreen negative materials, as well as «positives» were evaluated *in vivo*. The expense of performing all those *in vivo* tests would prohibit most laboratories (including a repeat of such work by us) from accumulating the sample sizes available in the present analysis.

In vitro cytotoxicity data obtained using seven human and murine tumor cell lines were correlated with *in vivo* antitumor data using P388 leukemia or B16 melanoma murine tumor models. The samples tested were comprised of hundreds of complex fermentation extracts and fractionations, and, in smaller separate analyses, pure synthetic and natural products. Criteria for positivity and activity have been addressed in the Materials and Methods section.

With respect to the fermentation samples and their effect against ip P388, did the *in vitro* cell lines, considered individually, predict for the *in vivo* results? Frequency tables (data not presented) prepared for each cell line demonstrated a clear relationship between *in vitro* positivity and *in vivo* activity; chi-square tests showed there were highly significant ($p < 0.0001$) relationships in each instance. Additionally, Staquet *et al* (23) described a guideline for a practical prescreen; namely, that the TPR associated with the prescreen should exceed 1-TNR (or else the likelihood of selecting an *in vivo* active sample is no better for an *in vitro* positive sample than an *in vitro* negative sample).

Each of the cell lines yielded TPRs which exceeded both 1-TNR and also the underlying prevalence associated with the sample tested.

Did the cell lines differ with respect to how well they predicted for P388 activity among fermentation samples? The selection of the «best» cell line for screening depends, we feel, on achieveing a reasonable balance between TPR, TNR and sensitivity. If one were concerned only with TPR and was unhappy with using Moser cells at a 1-256 dilution positivity criterion (TPR was 42%), one needn't replace Moser with another cell line, but instead, only increase the stringency of the positivity criterion to a 1-1,024 dilution (TPR of 51%). But choosing a cell line on the basis of only TPR is to ignore many other facets of screen selection. For example, Colon 26 cells used at an IC50 cutoff of 1-1,024 dilution had a TPR of 53%, but the sensitivity was a dismal 29%. If the aim of *in vitro* screening is to detect as many *in vivo* actives as is practically possible (sensitivity with yield taken into consideration), as well as accurately predict *in vivo* actives (TPR) and *in vivo* inactives (TNR), one must balance several correlation parameters.

In an attempt to determine if any one of the cell lines used in our screening procedures was superior to the other cell lines, we decided to compare them at positivity criteria which generated similar yields among samples of nearly identical prevalences. By doing so, we eliminated the possiblity that a cell line might appear to be a better screen soley because of greater innate susceptibility to cytotoxic agents. When analyzed at similar yields, rather than any single IC50 dilution, HCT-116 cells performed better than any other cell line we evaluated (with respect to fermentation samples and *in vivo* P388 activity). In several instances, usually dependent upon having an adequate sample population, the advantage of using HCT-116 cells was supported with differences of statistical significance. Despite the finding of a mild cell line superiority accompanied by occasional comparisons of statistical significance, the overall impression gleaned was one of comparability between the cell lines.

Would the correlations between *in vitro* cytotoxicity data

Table V. *Evaluation of* in vitro *cytotoxicity data and* in vivo *P388 leukemia data for chemically-defined compounds: Effect of varying positivity criteria*[a].

Cell line	*In vitro* IC50 (μg/ml)	No. of comparisons	Yield	PREV	TPR	TNR	SENS
KB	100	242	.52	.48	.78	.84	.84
	25	»	.45	.48	.83	.77	.72
	10	»	.34	.48	.85	.71	.60
HCT-116	100	144	.56	.56	.91	.89	.91
	25	»	.48	.56	.93	.79	.80
	10	»	.42	.56	.93	.72	.71
Moser	100	285	.42	.48	.83	.77	.73
	25	»	.35	.48	.89	.70	.60
	10	»	.26	.48	.88	.65	.47
RCA	100	140	.54	.56	.82	.75	.79
	25	»	.40	.56	.91	.68	.65
	10	»	.32	.56	.98	.64	.56
C26	100	270	.51	.47	.78	.85	.84
	25	»	.40	.47	.83	.77	.70
	10	»	.35	.47	.86	.74	.65
B16-F10	100	293	.45	.48	.80	.77	.74
	25	»	.33	.48	.86	.70	.58
	10	»	.27	.48	.87	.66	.48
M109	100	275	.45	.45	.80	.82	.78
	25	»	.32	.45	.83	.72	.58
	10	»	.25	.45	.87	.69	.49

(Parameters calculated: Yield, PREV, TPR, TNR, SENS)

[a]Activity *in vivo* versus P388 was based on a T/C of ≥ 125%.

and *in vivo* activity data improve if two cell lines were used in concert (rather than any one cell line)? In performing this series of comparisons, only subsets of fermentation samples tested in common by each member of a cell line pair under scrutiny were analyzed. The first manner in which this type of analysis was perfomed was to use a rule which, briefly stated, allowed a positive result *in vitro* obtained with *either* of two cell lines to be considered as a positive *in vitro* result in general.

All 21 possible two-cell line combinations among the seven cell lines used were evaluated. The TPR, TNR and sensitivity values resulting from the use of any two cell lines were not usually better than the values obtained using only the better cell line contained in each cell line pair. If there were any occasional improvements associated with the use of a particular cell line pair versus its individual cell line constituents, it was refelected by slight improvements in sensitivity. One triplet-cell line combination was also evaluated (data not presented), HCT-116, B16-F10 and Moser, but the TPR (44%), TNR (86%) and sensitivity (78%) found for it was not meaningfully better than those associated with HCT-116 cells alone.

Would the ability to predict for *in vivo* P388 activity improve if instead of «either cell line» in a pair being used as the benchmark for establishing *in vitro* positivity, *both* cell lines had to yield a positive response *in vitro* before the effect of the samples was deemed positive? We found that for every cell line pair evaluated, the majority of TPs was defined in common by both cell lines. Intuitively, one would expect that combining two of the better cell lines (those yielding the highest individual TPRs), e.g. HCT-116 and RCA cells, and imposing the aforementioned «both cell lines» rule for positivity *in vitro*, would yield a very good cell line pair-TPR. This expectation is made even more attractive given the added information that of all the *in vitro* positive samples so designated by these two cell lines, 86% of them were found to be positive by both HCT-116 and RCA cells. Surely such unanimity would portend well for a high TPR. Yet we found that only a 51% TPR was obtained, and this represented only a minor insignificant improvement over the TPRs associated with HCT-116 or RCA alone. Generally, the TPRs obtained using two cell lines in concert were similar to the TPRs associated with each cell line considered individually. Therefore, there would be no reason, on the basis of TPRs, to utilize a second cell line for the purpose of confirming an already obtained *in vitro* positive result.

Although two cell lines may yield similar individual TPRs, perhaps they are predicting for different subpopulations in the

Table VI. *Correlation of* in vitro *cytotoxicity data and* in vivo *B16 melanoma data for fermentation materials using single and multiple cell line analysis*[a].

Cell line(s)	n	Parameters calculated				
		Yield	PREV	TPR	TNR	SENS
HCT-116	125	.41	.37	.53	.74	.59
Moser	128	.49	.37	.52	.79	.70
B16-F10	147	.46	.39	.50	.70	.57
HCT-116 & Moser	121	.52	.36	.49	.79	.72
HCT-116 & B16-F10	125	.57	.37	.44	.73	.67
Moser & B16-F10	127	.61	.37	.49	.82	.81
HCT-116 & Moser & B16-F10	121	.61	.36	.46	.81	.79

[a]IC50 values of 1-1,024 for Moser and B16-F10, and 1-4,096 for HCT-116 were used as criteria for positivity *in vitro*. A positive *in vitro* result when using more than one cell line was construed, for the purpose of this analysis, as an IC50 obtained with *either* (or any) cell line which met the specified criteria.

total sample. This question of discordance, or extent of individuality (uniqueness), between cell lines was also analyzed. An assessent of how often unique predictions of *in vivo* activity were made by each cell line can be gleaned by comparing the incidence of unique TPs (and the unique TPR) versus the incidence of TPs obtained by both cell lines in common (and the TPR of each cell line without regard to the other paired cell line). In some cell line pairs, e.g. KB plus HCT-116 cells, most of the unique TPs were claimed by HCT-116 cells and the unique TPR associated with these cells was superior to the unique TPR found with the KB cells and mildly (insignificantly) better than the TPR for HCT-116 determined without regard to KB cell predictions. Thus, in this pairwise example, the addition of one cell line (HCT-116) contributed most of the unique predictions of *in vivo* activity (TPs) and did so in a much more accurate manner (TPR) than the other cell line (KB).

Sometimes however, as in the pair Moser plus RCA cells, most of the unique TPs were predicted by one of the cell lines, RCA, but the TPR for RCA-only positive samples was no better than the TPR for Moser-only positive materials. The point to be made here is that unique predictions need not be any more accurate than commonly made predictions, or vice versa. We found that the cell lines which yielded the best correlation parameters (particularly TPRs) when considered on an individual basis were sufficient for purposes of screening without the added cost (for minimal benefit) of including another cell line. Using the data from any two cell lines did not improve the acuity of the prescreen beyond that associated with the better cell lines used singularly.

It is conceivable, although unlikely, that of the hundreds of fermentation samples screened, only a handful of different antitumor chemotypes were represented. If true, the correlation parameters determined for the *in vitro* cell line data and *in vivo* P388 data could be relevant for only those limited number of chemotypes and not microbially-derived fermentation products in general. But the correlation analyses performed on the data generated from testing chemically-defined materials would support the ubiquitous utility of the *in vitro* cell line assay as a tool for predicting P388 activity. The chemically-defined materials contained in our assays represented a diverse collection of natural and synthetic compounds which included many of the known clinically active antitumor agents and their unproven analogs. Thus, while it may be worthwhile to raise the possibility that the fermentation samples we evaluated represented a limited range of chemotypes (we won't know their identities for some time, if ever), the demonstrated utility of the *in vitro* prescreen when applied to chemicall-defined compounds would suggest the approach is useful for many chemotypes.

With respect to fermentation samples and the correlation between *in vitro* cytotoxicity and *in vivo* B16 activity, our conclusions are admittedly based on a relatively limited sample size. Furthermore, the samples subjected to *in vivo* B16 testing often had shown some measure of cytotoxicity (IC50 of ≥ 1-256) versus some cell line and/or had failed to demonstrate activity *in vivo* versus P388. Nevertheless, while practical limitations caused us to evaluate a selected sample opulation, we have no reason to suspect that this sample has introduced a bias in the results. Only future application of *in vitro* test results with *in vivo* B16 testing amongst an unselected sample of fermentations, followed by a comparison with our own correlation data, will permit a possible resolution of the question of bias.

We wished to know which cell line best detected and predicted for *in vivo* B16 activity and we limited our prescreening to Moser, HCT-116 and B16-F10 cells. With extremely similar prevalences and reasonably similar yields, each cell line produced equivalent TPRs. Ratios of TPR/prevalence ranged from 1.28 to 1.43 and illustrate the comparability of the

Table VII. *Extent of accordance and discordance among selected cell lines with respect to correlations between* in vitro *cytotoxicity and* in vivo *B16 melanoma data for fermentation materials*[a].

Cell lines:			Incidence (% of total category)						% TPR				
A	B	n	True positives			False positives			A&			A+	B+
			Common	Unique A	Unique B	Common	Unique A	Unique B	B	A	B	B−	A−
HCT-116	Moser	121	26(84)	0 (0)	5(16)	20(63)	3 (9)	9(28)	57	53	52	0	36
HCT-116	B16-F10	125	23(74)	4(13)	4(13)	17(45)	7(18)	14(37)	58	56	47	36	22
Moser	B16-F10	127	26(68)	7(18)	5(13)	23(58)	7(18)	10(25)	53	52	48	50	33

[a]Positivity *in vitro* was defined as an IC50 of 1-1,024 for Moser and B16-F10 cells, and 1-4,096 for HCT-116.

cell lines in predicting for *in vivo* B16 activity. The Moser cell line had a higher sensitivity and TNR than the two other cell lines, at the *in vitro* positivity levels applied, but the differences were not statistically significant with the sample sizes available.

Data from more than one cell line were analyzed collectively to prescreen fermentation samples for *in vivo* B16 testing using the rule that a positive result *in vitro* with *either* of two cell lines (or *any one* of the three cell lines) was considered a positive *in vitro* result. None of the pairwise combinations, nor all three cell lines together, provided better correlation parameters than the better (or best) cell line in a combination. The TPRs associated with the combinations were actually reduced from each single cell line's TPR. The only noticeable improvement attributed to merging of two or more cell lines' data was found in the sensitivity parameter. But this might have been expected given the higher yields associated with these combined data sets.

Instead of defining *in vitro* positivity for two or more cell lines as we did in the preceding paragraph, we also defined it by insisting that *both* (or *all*) cell lines yield IC50 data consistent with positivity. When the correlation data were analyzed by this determinant we observed that each pairwise combination defined in common the majority of TP samples tested. Furthermore, the commonly derived TPRs were similar to the individually derived TPRs associated with the better (more predictive) cell line in each pair. Thus, there would be no reason, on the basis of TPR, to utilize a second cell line for the purpose of confirming an already obtained *in vitro* positive result.

Only a minority of fermentation samples were defined as positive *in vitro* by one cell line but not the other in pairwise comparisons. In every evaluation made of these unique detections, there were as many or more FPs as TPs and thus the unique TPRs never exceeded 50%. In practical terms, it would not appear to be cost effective to prescreen with two cell lines (instead of one) for the purpose of salvaging the few samples uniquely predicted for *in vivo* activity by one (but not the other) of the cell lines. Nevertheless, despite the preponderance of commonality of discovery, the desire to find *in vivo* B16 active materials may influence prescreen design in a manner consistent with improved ability to detect such materials. Accordingly, the 16-18% of the TPs uniquely found by Moser cells (or 13% of the TPs found only by B16-F10 cells) may be worth the cost of including more than one cell line in certain prescreen assays.

With the sample populations available, there was no statistical advantage discerned to justify recommending between HCT-116, B16-F10 and Moser for predicting for ip B16 antitumor activity. At least twice the sample would have to be evaluated in each of the cell lines and ip B16 before the magnitude of the larger differences in correlation parameter values observed became statistically noteworthy.

Based on a limited data base, but one we feel will reflect the likely outcome of more extensive investigations, the cell line cytotoxicity assay utilizing HCT-116, Moser and B16-F10 cells was not a useful prescreen for detecting and predicting fermentation samples having the ability to increase the lifespan of mice implanted sc with B16. Yet for ip B16 and ip P388 *in vivo* tumor models, the *in vitro* cytotoxicity assay has provided a useful prescreen for selecting *in vivo* active materials.

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Clinical Predictive Value of the *in Vitro* Cell Line, Human Xenograft, and Mouse Allograft Preclinical Cancer Models[1]

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ABSTRACT

Purpose: **We looked at the value of three preclinical cancer models, the *in vitro* human cell line, the human xenograft, and the murine allograft, to examine whether they are reliable in predicting clinical utility.**

Experimental Design: **Thirty-one cytotoxic cancer drugs were selected. Literature was searched for drug activity in Phase II trials, human xenograft, and mouse allografts in breast, non-small cell lung, ovary, and colon cancers. Data from the National Cancer Institute Human Tumor Cell Line Screen were used to calculate drug *in vitro* preclinical activity for each cancer type. Phase II activity *versus* preclinical activity scatter plot and correlation analysis was conducted for each model, by tumor type (disease-oriented approach), using one tumor type as a predictor of overall activity in the other three tumor types combined (compound-oriented approach) and for all four tumor types together.**

Results: **The *in vitro* cell line model was predictive for non-small cell lung cancer under the disease-oriented approach, for breast and ovarian cancers under the compound-oriented approach, and for all four tumor types together. The mouse allograft model was not predictive. The human xenograft model was not predictive for breast or colon cancers, but was predictive for non-small cell lung and ovarian cancers when panels of xenografts were used.**

Conclusions: **These results suggest that under the right framework and when panels are used, the *in vitro* cell line and human xenograft models may be useful in predicting the Phase II clinical trial performance of cancer drugs. Murine allograft models, as used in this analysis, appear of limited utility.**

INTRODUCTION

Both basic science studies and clinical trials are essential components of the cancer drug discovery process. Potential therapeutics found to be significantly better than no treatment or standard therapies (*i.e.*, active) in preclinical laboratory cancer models or compounds with novel chemotypes and equivalent effectiveness to standard treatments are advanced to confirmatory testing in early (Phase I and II) clinical trials. Considering that RR[3] is a reasonable surrogate end point for survival (required but not sufficient), a favorable RR in Phase II trials advances a drug into additional clinical testing and is considered a prerequisite of drug success in the clinic.

Advancing of a candidate drug from preclinical testing in the laboratory to testing in Phase II clinical trials is based on the assumption that drug activity in cancer models translates into at least some efficacy in human patients, *i.e.*, that cancer laboratory models are clinically predictive. In addition, the relevance of tumor type-specific preclinical results for the corresponding human cancers in the clinic can be viewed through two different approaches: compound-oriented, where a drug is assumed to have potential activity against all human tumor types if it is effective against a single test tumor type, and disease-oriented, where a drug with preclinical activity in a single tumor type would only be expected to be effective in the same tumor type in patients.

Although widely adopted, the above-mentioned assumption and approaches have not been confirmed by studies to date. In addition, all studies aimed to examine the clinical predictive value of laboratory cancer models inevitably suffer from inherent bias because compounds with no activity in preclinical models are generally not advanced to clinical trials.

This work was undertaken to examine the clinical predictive value of three preclinical cancer models that have found wide use: the human *in vitro* cell line; the mouse allograft; and the human xenograft. In these models, tumor volume or life span (*in vivo* mouse models) or cell growth (*in vitro* cell lines) is compared between the treatment group receiving the new drug and a control group (active or inactive control).

The use of preclinical cancer models for selection of potential cancer therapeutics was pioneered by the NCI in the United States in the mid-1950s. The screening strategies used until 1990 were essentially compound oriented and involved a

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[3] The abbreviations used are: RR, response rate; NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; NSC, National Service Center; T/C%, treated over control tumor volume ratio.

small number of predominantly murine allograft tumors, with emphasis on leukemia (1–7). Several studies from the NCI and others demonstrated that this approach had low clinical predictive value for activity in Phase II trials (5–9) and yielded compounds with selective activity toward human leukemias and lymphomas (10–12). Thus, in 1990, the NCI introduced a disease-oriented *in vitro* Human Tumor Cell Line Screen comprised of 60 cell lines from the most common adult tumors (13–17). The screen was designed so that each tumor type was represented by a panel of cell lines, selected on the basis of different subhistological features, and common drug resistance profiles. It was hoped that this screen would help identify drug leads with high potency and/or selective activity against particular tumor types.

Recently, the NCI examined the correlation between drug activity in Phase II clinical trials and preclinical activity in cancer models (18). Important findings were: (*a*) with the exception of NSCLC, preclinical activity in human xenografts of a particular tumor type did not correlate significantly with Phase II activity in the same type of tumor, (*b*) with the exception of breast and colon histologies, human xenografts did not significantly predict Phase II clinical activity in other cancers types; and (*c*) compounds that were active in at least one-third of all tested human xenografts were likely to have at least some activity in Phase II clinical trials.

Studies examining the clinical predictive value of preclinical cancer models outside the scope of the NCI screening programs have focused on the human xenograft model and have looked predominately into same-tumor correlations (disease-oriented approach). These studies have produced both positive (the model was found clinically predictive) and negative (the model was found to have no clinical predictive value) results in various tumor types (19–27).

Two major criticisms can be made on the overall body of literature concerning the clinical predictive value of preclinical cancer models. First, the vast majority of studies to date, both within and outside the NCI, have based their conclusions on the observation of trends rather than the use of statistical methods. Second, all studies conducted previously have used dichotomous definitions of preclinical and/or clinical activity based on largely invalidated cutoff values of measures of activity: a 20% RR in Phase II clinical trials and (most commonly) a 42% T/C% in human xenografts and mouse allografts.

In addition, two important questions have not been addressed at all by previous studies: the clinical predictive value of the *in vitro* cell line model and the relative clinical usefulness of the different preclinical cancer models in use today (*i.e.*, how different models compare with each other in terms of their ability to identify clinically effective drugs).

Thus, we conducted a study comparing the clinical (Phase II) predictive value of three widely used preclinical laboratory cancer models, the *in vitro* human cell line, the mouse allograft, and the human xenograft. We used quantitative measures of both clinical and preclinical activity and statistical methods. We considered three relevant questions: (*a*) the clinical predictive value of the three models within the same tumor type (disease-oriented approach); (*b*) the clinical predictive value of the three models when one preclinical tumor type is used as a predictor of overall clinical activity in all other tumor types (compound-oriented approach); and (*c*) the clinical predictive value of the three models when overall preclinical and clinical activity in all tumor types combined is considered.

MATERIALS AND METHODS

Study Design

A retrospective, literature-based study was conducted. Data were retrieved from studies published between 1985 and 2000. This period was chosen as one when all three preclinical cancer models of interest to this study were in use and because it was long enough and close enough to the present as to afford data on a relatively large number of recently developed drugs.

The data search was restricted to four of the most common and commonly studied solid tumor types, breast, colorectal, ovarian, and non-small cell lung cancers, to ensure that sufficient data would be available.

The Medline and CancerLit databases were used for the collection of published data. In an attempt to minimize publication bias, both paper publications (peer reviewed) and meeting abstracts (nonpeer reviewed) were used as sources of information. If published data were not available for identified drugs, manufacturers were contacted for unpublished data.

Selection of Drugs

Drugs were identified by searching the Medline and CancerLit databases for compounds that had undergone single agent Phase I clinical trial testing either in 1991 or 1992. Agents with novel targets such as signal transduction or angiogenesis modulators were not included.

This Phase I-based approach to agent identification was used to ensure selection of agents developed within the study time frame of 1985–2000: agents with a published Phase I clinical trial in 1991 or 1992 were expected to have been through preclinical testing between 1985 and 1990 and to have undergone Phase II clinical evaluation by the year 2000. In addition, this approach was adopted to minimize publication bias: publication of Phase I trials is generally less dependent on the observation of favorable tumor responses than publication of Phase II trials or of preclinical cancer model experiments.

Data Collection and Drug Activity

Phase II Clinical Trials. Phase II clinical trials for each drug were identified by searching the Medline and CancerLit databases for scientific papers, reviews, or meeting abstracts. Duplicate publications were discarded. For trials with only abstract information, an additional search by author and/or institution name was conducted in Medline or CancerLit. Scientific papers were used in preference to abstracts, where possible.

Two restrictions were applied. The first was a geographic restriction: to ensure uniform methodology in trial conduct and RR assessment, only Phase II trials conducted in the Americas, Western Europe and Australia were included in the analysis. The second restriction referred to the treatment population and aimed to ensure that uniformly responsive populations of patients would be considered. For breast and ovarian cancer, only Phase II trials that included patients who had received prior chemotherapy for metastatic disease were used, whereas for

NSCLC and colon cancers, the Phase II trials selected included patients who had received no prior chemotherapy.

For each individual Phase II trial the following information was collected: disease site; previous chemotherapy; disease stage; number of patients entered; eligible; evaluable and evaluable for response; number of complete and partial responses; and criteria used for response (standard WHO *versus* other). Trials had to have enrolled a minimum of 14 patients, at least 12 of whom must have been evaluable for response. Completed Phase II trials for which >20% of entered patients were listed as inevaluable for response were considered methodologically unacceptable and were not used. For trials in progress at the time of reporting (meeting abstract format only), the available data were used even if they represented <80% of the enrolled patients, provided that they met the 14-patient criterion. If a trial publication did not specify the previous chemotherapy treatment status of patients, it was not used. Information from Phase I-II trials was used only when the Phase I and II components of the trial were separately conducted and reported. Phase II information was collected regardless of drug dose and route of administration.

For a given drug, in a given cancer type, the activity in a single Phase II clinical trial was recorded as the RR: the number of partial and complete tumor responses over the total number of patients evaluable for response. The number of evaluable rather than eligible patients was used to accommodate information from trials for which final results were not available. In the very few cases where the number of patients evaluable for response was not provided, the number of evaluable patients, the number of eligible patients, or the number of patients entered in the trial (whichever was provided by the investigators) in that priority order was used.

To obtain a drug's overall clinical activity in multiple Phase II trials of patients with the same tumor type, all responses and the collective number of patients evaluable for response were pooled from individual trials to calculate an overall RR. Finally, to get the Phase II activity for any three or four cancer types combined, the individual tumor RRs were averaged.

Human Xenografts and Mouse Allografts. The search strategy for mouse cancer model data were similar to the Phase II process. The only exclusion in this case were results obtained with mouse tumors that were engineered to have special characteristics such as, for example, overexpression of proteins conferring drug resistance.

For each murine allograft or human xenograft, numerical value(s) of activity for drugs of interest was retrieved only if expressed as the treated over control tumor volume ratio (T/C%) or the tumor volume growth inhibition ratio (GI%; and T/C% = 100% − GI%) in the literature sources. In addition, only T/C% values calculated by the formula T/C% = [(RVtreated)/(RVcontrol)] × 100% were collected (where RV = relative volume), whereas T/C% values defined for regressions [T/C% = [(RVtreated (0) − RVtreated(t))/RVtreated (0)] × 100%] were excluded to ensure uniform calculation methods. If the T/C% was not provided but a relative tumor growth curve was given as a figure in a publication, the numerical values for the treatment and control groups provided in this graph were used to calculate the T/C%. Activity reported as all mice cured or 100% complete responses was considered equivalent to and recorded as a T/C% = 0. If no exact T/C% value was given but an interval of values was provided instead (*i.e.,* T/C% >42), a T/C% equal to the interval midpoint value (*i.e.,* a T/C% = 71) was assigned. Finally, where preclinical activity was reported as GI%, it was converted to T/C% by the formula T/C% = 100% − GI%. The activity value for the most effective, nontoxic dose in each schedule was recorded.

Single tumor type preclinical activity of each drug in the murine allograft or human xenograft models was defined as the mean T/C% value from all tested allografts/xenografts of that tumor type. Where the same laboratory had tested a single xenograft/allograft with multiple schedules of the same drug and/or where the same xenograft/allograft had been tested with the same drug by more than one laboratories, T/C% values for a single tumor were obtained by first averaging the same laboratory T/C% values and then the same xenograft T/C% values.

Overall preclinical activity in xenografts/allografts for all four tumor types together was expressed as the average of single tumor mean T/C% values.

***In Vitro* Human Tumor Cell Lines.** The publicly available data from the NCI's Human Tumor Cell Line Screen was used as the information source for the *in vitro* tumor cell line model. Information from the NCI *in vitro* Human Tumor Cell Line Screen was favored because it was a readily available, well-defined, comprehensive, validated, and extensive single source of data. Another important reason was that as an exploratory literature search showed, there was such a wide variation between different investigators in the types of assays used and the nature of cell lines tested that it would have been impossible to comprehensively combine published data from various laboratories.

Acquisition of NCI Human Tumor Cell Line Screen data were done through the internet.[4] Information for each drug was obtained through its NCI code number or NSC number. Such numbers, where available, were identified either from the literature or from a cross-reference of compound names and NSC numbers in the NCI database (also available on the NCI web site).[4]

Testing of compounds in the NCI *in vitro* Human Tumor Cell Line Screen has been described previously (17). Briefly, growth inhibition in cell lines is measured by the GI_{50}, defined as the drug concentration that causes a 50% reduction in cell number in test plates relative to control plates. For every drug entering the screen, a concentration range comprised of five, 10-fold dilutions is tested in each of a group of 60–80 cell lines. The optical densities between treated and control plates, as resulting from the sulforhodamine B assay, are used to construct a dose-response curve for each cell line in the screen, leading to the calculation of a GI_{50} in every case by interpolation. In the case of compounds with low (*i.e.,* the highest concentration tested causes <50% growth inhibition) or high (*i.e.,* the lowest concentration tested causes >50% growth inhibition) potency where interpolation is not possible, the highest and lowest concentrations, respectively, in the tested drug concentration

[4] Internet address: http://www.dtp.nci.nih.gov/docs/cancer/searches/cancer_open_compounds.html.

range are recorded as the approximated GI_{50}s. GI_{50}s are then converted to their Log_{10} values and the overall mean $Log_{10}GI_{50}$ across all cell lines in the screen is calculated. Finally, the results are displayed by a bar graph called the mean graph (28). This graph lists all of the cell lines and their corresponding $Log_{10}GI_{50}$s and relates the magnitude of every individual cell line $Log_{10}GI_{50}$ to the mean $Log_{10}GI_{50}$ across all of the cell lines by a bar to the right (more sensitive than average) or to the left (less sensitive than average) of a vertical line. The experiment is repeated several times for each concentration range. In cases where mean graphs are based on mostly approximated GI_{50}s, other higher or lower concentration ranges of the drug (again made of five, 10-fold dilutions) are also tested. Thus, for each compound tested in the NCI *in vitro* Human Tumor Cell Line Screen, multiple GI_{50} mean graphs (one for each concentration range tested) based on multiple experiments each and with a different content of approximated *versus* calculated (by interpolation) GI_{50}s may exist in the NCI database.

We obtained all of the available GI_{50} mean graph information from the NCI web site for all drugs in our list of compounds with known NSC numbers.[4] For every drug, we recorded the number of concentration ranges tested in the NCI *in vitro* Human Tumor Cell Line Screen, the number of experimental repetitions conducted for each concentration range, and, finally, the number of approximated $Log_{10}GI_{50}$s in each mean graph.

The drug concentration range that produced the mean graph with the smallest number of approximated $Log_{10}GI_{50}$s was used for scoring a drug's activity in the NCI *in vitro* Human Tumor Cell Line Screen, unless a different concentration range existed, with a number of approximated $Log_{10}GI_{50}$s varying <10% from the first but for which more experiments were done.

Preclinical activity in the NCI *in vitro* Human Tumor Cell Line Screen was scored in two different ways: by the mean $Log_{10}GI_{50}$ and by what was termed the activity fraction. For a given drug, in a given tumor type, the mean $Log_{10}GI_{50}$ was computed by averaging the $Log_{10}GI_{50}$s from all of the cell lines of that tumor type in the mean graph corresponding to the most appropriate concentration range. The activity fraction was arbitrarily defined as the number of cell lines of a given tumor type in which the individual $Log_{10}GI_{50}$s were more sensitive to the drug than the average $Log_{10}GI_{50}$ (for all cell lines of all cell types) in the mean graph over the total number of cell lines tested from that tumor type. The activity fraction was also calculated from the mean graph corresponding to the most appropriate concentration range. Overall mean $Log_{10}GI_{50}$s or activity fractions for all four cancer types combined were calculated by averaging the single tumor values.

Table 1 Drugs selected for data collection. NSC numbers are shown, where available

Drug	NSC number
Taxotere	628503
Paclitaxel	125973
Topotecan	609699
Irinotecan	
Rhizoxin	332598
Gemcitabine	
Fazarabine	281272
Teniposide	122819
Menogaril	269148
Fosquidone	D611615
Elsamitrucin	369327
Amonafide	308847
Didemnin B	325319
Suramin	
Raltitrexed	639186
Flavone acetic acid	347512
Epirubicin	256942
CI-921	343499
Trimetrexate	352122
Multitargeted antifol	
Vinorelbine	
Piritrexim	351521
Fotemustine	
CI-980	
Chloroquinoxaline sulfonamide	339004
Ilmofosine	
CI-941	
Tiazofurin	286193
Pyrazine diazohydroxide	361456
Tallimustine	
Crisnatol	

Statistical Analysis

For each preclinical cancer model, 9 Phase II *versus* preclinical activity relationships were examined for a total of 27: relationships by tumor type (disease-oriented approach, 4 relationships/model), predictive ability of one tumor type for the other three tumor types combined (compound-oriented approach, 4 relationships/model), and general predictive ability for all four tumor types combined (1 relationship/model).

Relationships were first examined descriptively with the construction of various Phase II overall activity *versus* preclinical activity scatter plots (Microsoft Excel software). Each point on these scatter plots represented data from one drug for which both Phase II and preclinical activity values had been calculated from literature sources, as described above.

After descriptive evaluation of the data, Spearman rank correlation coefficients were obtained using the SAS software, UNIX version 6.12. A significance test of every correlation coefficient was performed, and the corresponding *P*s were calculated. Spearman rank (nonparametric) correlation coefficients were used because the distributions of the *x* (preclinical activity) and *y* (clinical activity) variables were not normal (29).

When multiple comparisons are made within a group of data such as in this work, there is increased possibility that some correlations will come up as statistically significant solely because of chance (false positives). To avoid this, multiple comparison correction methods (*e.g.*, Bonferroni approach) are often used to adjust the significance level to a lower *P* than conventionally used. However, relying on corrected probabilities increases the possibility that meaningful correlations will be missed (false negatives), making the nature of the scientific work key to the decision to use multiple comparison adjustment methods or not. Because this was an exploratory study, we were willing to accept a higher probability of false positives to ensure that potentially meaningful associations would not be discarded. We therefore did not correct for multiple comparisons and chose a level of significance of 0.05.

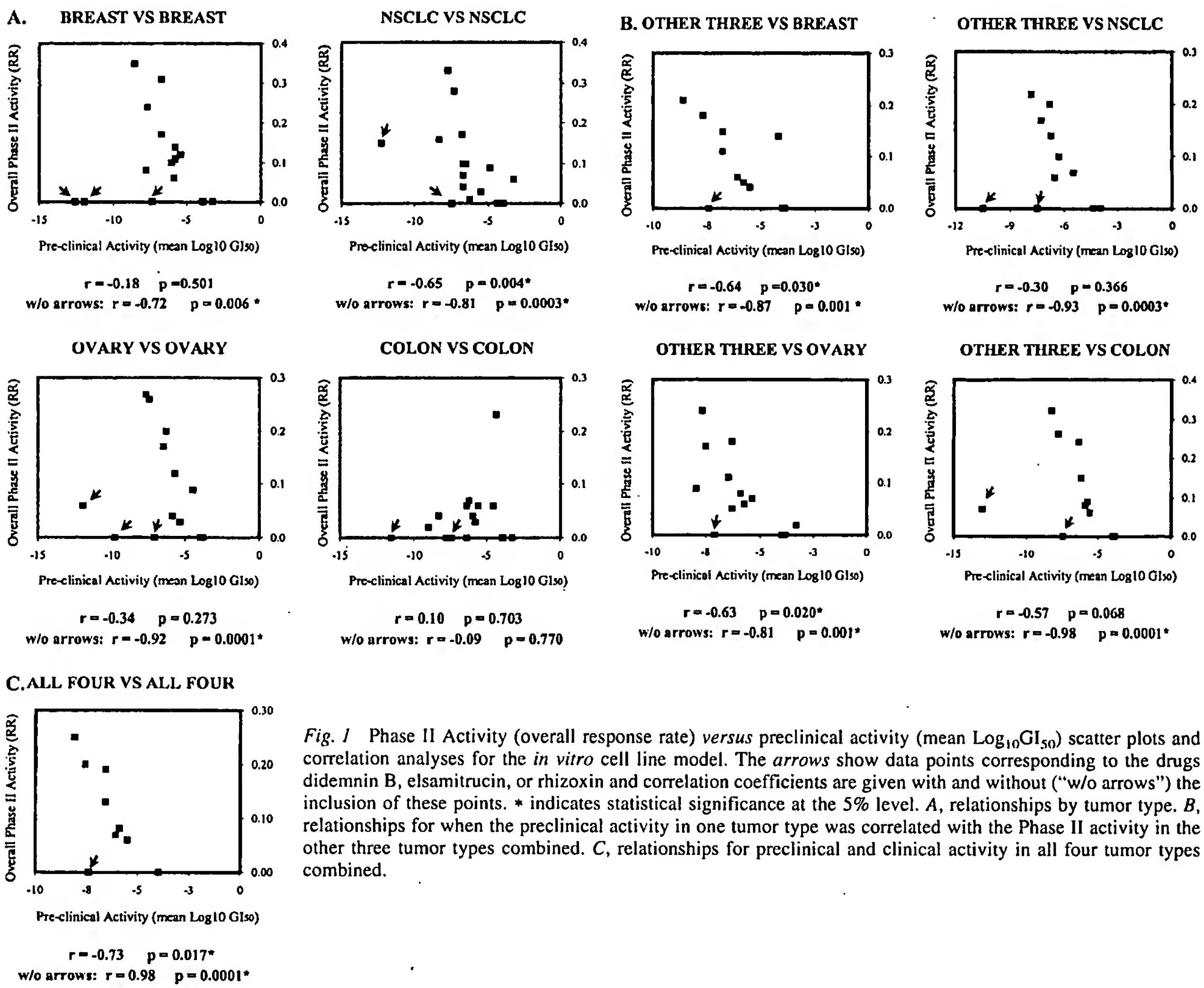


Fig. 1 Phase II Activity (overall response rate) *versus* preclinical activity (mean $Log_{10}GI_{50}$) scatter plots and correlation analyses for the *in vitro* cell line model. The *arrows* show data points corresponding to the drugs didemnin B, elsamitrucin, or rhizoxin and correlation coefficients are given with and without ("w/o arrows") the inclusion of these points. * indicates statistical significance at the 5% level. *A*, relationships by tumor type. *B*, relationships for when the preclinical activity in one tumor type was correlated with the Phase II activity in the other three tumor types combined. *C*, relationships for preclinical and clinical activity in all four tumor types combined.

RESULTS

The Medline and CancerLit databases were searched for cancer drugs (excluding agents with novel targets such as signal transduction or angiogenesis modulators) that had undergone single agent Phase I clinical trial testing either in 1991 or 1992. This search led to 97 drug names. After excluding drugs that were eliminated from additional clinical testing for practical reasons (for example difficulties with the drug formulation), drugs that were specifically developed for a certain type of cancer (as for example hormone-regulating compounds for breast cancer) and drugs that were still the subject of published Phase I studies in 1991 and 1992 despite already being licensed for human use before 1985, a list of 31 agents was obtained (Table 1). After applying the restrictions and criteria mentioned under "Materials and Methods," we extracted from the literature preclinical and Phase II activity information for those agents on four common cancer types, breast, NSCLC, ovary, and colon. Overall, 100 preclinical and 307 Phase II clinical literature references were used spanning the period between 1985 and 2000.

No preclinical data were found for 5 of the 31 drugs researched. Of the 26 drugs remaining, availability of preclinical and Phase II data varied, depending on which preclinical and clinical tumor(s) had been tested and published in each case. Thus, each of the relationships examined had a different number of data points as different subsets of drugs were included. The most data points for any relationship were 17. For six relationships, five or fewer data points were available (relationships with fewer than five data points were not included in the results presented below).

***In Vitro* Cell Line Model.** Fig. 1 shows the Phase II activity *versus* preclinical activity scatter plots and correlation analysis for the *in vitro* cell line model when the mean $Log_{10}GI_{50}$ was used as the measure of preclinical activity. Because the lower the mean $Log_{10}GI_{50}$, the higher the potency of a drug, a negative correlation between mean $Log_{10}GI_{50}$ and Phase II overall RR was expected if the model had a good clinical predictive value. Significant negative correlations were found for NSCLC (Fig. 1*A*), for breast or ovarian cell lines *versus* overall Phase II activity in the other three tumor types

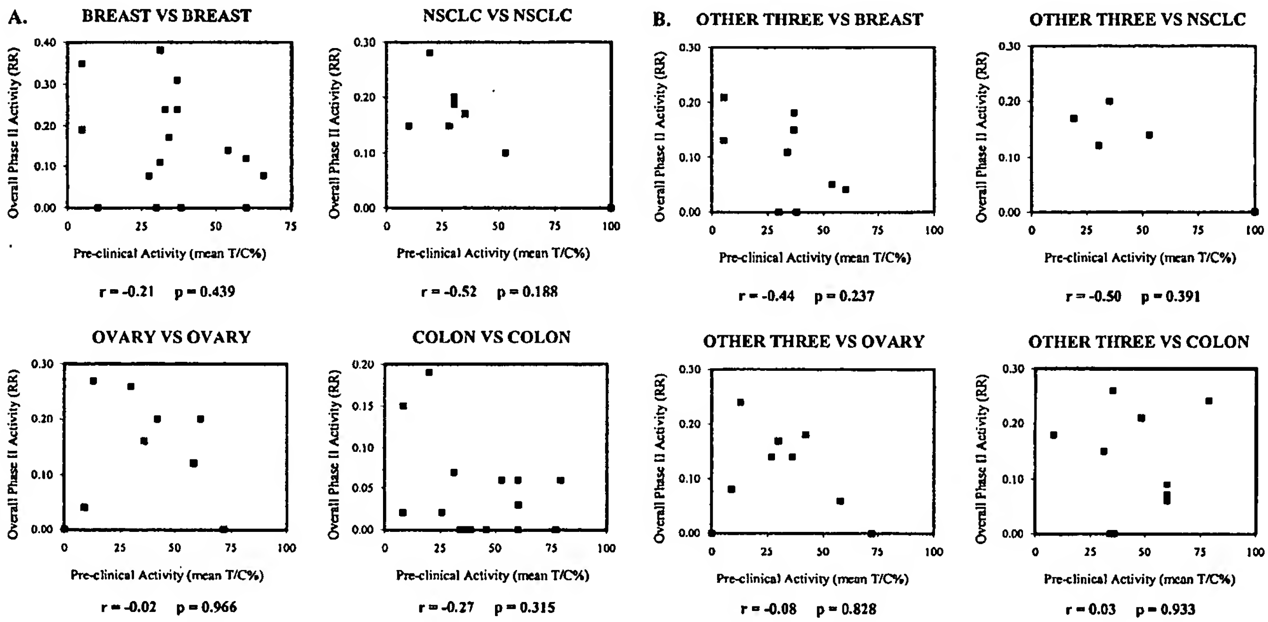


Fig. 2 Phase II activity (overall response rate) *versus* preclinical activity (mean T/C%) scatter plots and correlation analyses for the human xenograft model. *A*, relationships by tumor type. *B*, relationships for when the preclinical activity in one tumor type was correlated with the Phase II activity in the other three tumor types combined.

(Fig. 1*B*), and for preclinical activity *versus* Phase II activity in all four tumor types (Fig. 1*C*).

Although the trends observed with the activity fraction were similar to ones seen for the mean $Log_{10}GI_{50}$ measure, no correlations were statistically significant in this case (data not shown).

Human Xenograft Model. A negative correlation between Phase II RRs and mean T/C% values was expected to be indicative of a good clinical predictive value for the human xenograft model. As shown in Fig. 2, no significant correlations between preclinical and clinical activity were observed for this model in our analysis.

For some of the drugs, preclinical activity calculations were based on multiple human xenografts of the same tumor type (*i.e.*, panels) while for others on only a single xenograft. The relationships in Fig. 2 were reanalyzed, including only the drugs for which preclinical information on more than one human xenograft was available (Fig. 3). The results did not change for breast or colon tumors (compare Fig. 3*A* with Fig. 2*A*). However, the relationship for NSCLC became statistically significant and a highly significant correlation was seen for ovarian cancer (Fig. 3*A*). A near significant correlation was obtained when ovarian human xenograft panels were used to predict clinical activity in the other three tumor types combined (Fig. 3*B*).

Murine Allografts. No significant correlations between preclinical and clinical activity were observed for any of the relationships examined in this study for the murine allograft model (data not shown).

Additional Analyses. The scatter plots in Fig. 1 revealed an interesting observation: in every relationship except for colon cancer under the disease oriented approach, an obvious trend toward a negative correlation was evident except for one to three outlier data points (Fig. 1, *arrows*). Interestingly, in all cases, these outlier data points corresponded to the same three drugs, namely elsamitrucin, didemnin B, and rhizoxin.

In an attempt to provide a possible explanation for this observation, we considered the mechanism of action of all drugs that were included in the correlations in Fig. 1. From a total of 18 drugs (Table 2), 5, namely, elsamitrucin, didemnin B, rhizoxin, flavone acetic acid, and fosquidone, were distinct in that they seemed to act through mostly unknown pathways that were not the typical DNA-based mechanisms of action of cytotoxic cancer agents. Thus, although flavone acetic acid and fosquidone fitted the rest of the data, there seemed to be a plausible mechanistic basis for the outlier behavior of the data points for elsamitrucin, didemnin B, and rhizoxin. In fact, exclusion of these three drugs led to highly significant correlations in all cases except for the same-tumor relationship in colon cancer (Fig. 1, correlation coefficients and *P*s for "w/o arrows"). It should be noted that none of the relationships examined for the human xenograft models (Figs. 2 and 3) included elsamitrucin, didemnin B, or rhizoxin as data points.

Because of the intriguing results obtained with the human NSCLC and ovarian xenograft panels in Fig. 3*A*, a more detailed examination of these panels was pertained. As seen in Figs. 4*A* and 5*A*, the 6 ovarian and 7 NSCLC xenograft panels differed both in the numbers (minimum of 6 and maximum of 13 for ovary and minimum of 2 and maximum of 8 for NSCLC) and the identity of the xenografts that they contained. Analysis by grade/histology was hindered by lack of complete information on all xenografts. However, some patterns appeared distinguish-

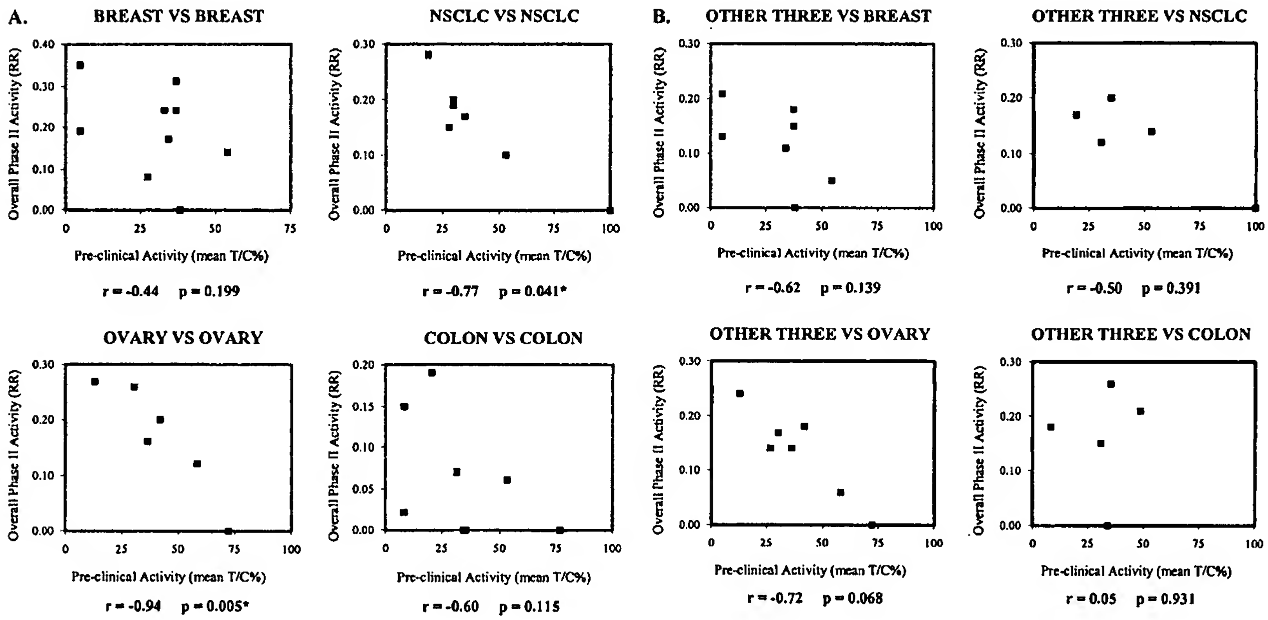


Fig. 3 Phase II activity (overall response rate) *versus* preclinical activity (mean T/C%) scatter plots and correlation analyses for the human xenograft model. Only data points for which two or more human xenografts were used to generate the preclinical activity values are shown. * indicates statistical significance at the 5% level. *A*, relationships by tumor type. *B*, relationships for when the preclinical activity in one tumor type was correlated with the Phase II activity in the other three tumor types combined.

Table 2 Mechanisms of action of drugs used in clinical vs. pre-clinical correlations for the *in vitro* cell line model (Fig. 1) Atypical cytotoxics are shown in bold.

Drug	Mechanism of action
Amonafide	DNA intercalator
CI-921	Acts on topoisomerase II
Didemnin B	Not understood. Believed to act on protein synthesis
Elsamitrucin	Not understood. It has been observed to inhibit topoisomerase I and II in *in vitro* experiments (relevance to *in vivo* uncertain). In cells in culture it has been observed to cause a cytostatic effect.
Epirubicin	Attaches to DNA at G bases
Fazarabine	Probably inhibits DNA synthesis by incorporation into DNA.
Flavone acetic acid	Has antivascular action in mice (probably not applicable to humans). Also believed to induce cell cycle arrest by generating reactive oxygen species that act on DNA.
Menogaril	Causes cleavage of double-stranded DNA by inhibiting topoisomerase II
Piritrexim	Inhibits dihydrofolate reductase
Rhizoxin	Not fully understood. May interact with tubulin (different binding site than taxoids) and lead to cell cycle arrest. Also observed to act as an angiogenesis inhibitor.
Taxol	Microtubule destabilizing agent that causes apoptosis
Taxotere	Microtubule destabilizing agent that causes apoptosis
Teniposide	DNA synthesis inhibition by stabilization of cleavable DNA complexes
Topotecan	Topoisomerase I inhibitor
Trimetrexate	Antifolate
Fosquidone	Unknown. Not a DNA binder or a topoisomerase inhibitor
Tomudex	Thymidylate synthase inhibitor
Tiazofurin	Inhibits 5′-phosphodehydrogenase, the rate-limiting enzyme for guanine ribonucleotide synthesis

able. All ovarian panels contained 10–20% undifferentiated tumors and also included both poorly differentiated and moderately differentiated subtypes (Fig. 4*B*). For NSCLC, all panels included adenocarcinoma xenografts with a frequency of >30% (Fig. 5*B*). These observations suggested that the frequency of histological/grade subtypes within a xenograft panel may be an important determinant of clinical predictivity rather than the number or the nature of the xenografts.

In an attempt to explore this hypothesis and to further examine the validity of the results obtained for ovarian cancer and NSCLC in Fig. 3*A*, the literature was reviewed for additional data. Six more agents with known overall Phase II RRs in

A.

NAME	HISTOLOGY / GRADE	DATA POINTS (DRUGS)					
		EPIRUBICIN	FOSQUIDONE	GEMCITABINE	MENOGARIL	TAXOTERE	PACLITAXEL
MRI-H-207	undifferentiated	+	+		+	+	
A2780	undifferentiated	+		+	+		+
Ov.He	mod. diff., mucinous	+	+	+	+		
Ov.Me	carcinosarcoma	+	+		+		
OvRiC	mod. diff., serous	+	+	+	+		
Fma	poorly diff., mucinous	+	+		+	+	
Ov.Pe	mod. diff., mucinous	+	+	+	+	+	
Fco	clear cell sarcoma	+	+		+		
T17	cystoadenocarcinoma	+					
T385	adenocarcinoma	+					
OvGR	mod. diff., mucinous		+				
Fko	mod. diff., serous		+	+		+	
OvG1	poorly diff., serous		+				
OVCAR-3	adenocarcinoma			+		+	+
A121a	?					+	+
HOC18	poorly diff., serous					+	+
HOC22	poorly diff., serous					+	+
A2780/DDP	undifferentiated						+
A2780/DX	undifferentiated						+
SKOV-3	adenocarcinoma						+
1° ovary 1	cystoadenocarcinoma						+
1° ovary 2	dediff. serous adenoc.						+
IGROV 1	moderately diff.						+
OVCAR-8	poorly diff. adenoc.						+
OVCAR-5	adenocarcinoma						+
OvSh	poorly diff., serous					+	
HOC22-S	poorly diff., serous					+	
TOTAL NO.		10	10	6	8	10	13

B.

HISTOLOGY/GRADE FREQUENCIES IN HUMAN OVARIAN XENOGRAFT PANELS						
HISTOLOGY / GRADE	EPIRUBICIN NO. (%)	FOSQUIDONE NO. (%)	GEMCITABINE NO. (%)	MENOGARIL NO. (%)	TAXOTERE NO. (%)	PACLITAXEL NO. (%)
undifferentiated	2 (20)	1 (10)	1 (17)	2 (25)	1 (10)	3 (23)
mod. diff., mucinous	2 (20)	3 (30)	2 (33)	2 (25)	1 (10)	0 (0)
mod. diff., serous	1 (10)	2 (20)	2 (33)	1 (12.5)	1 (10)	0 (0)
poorly diff., mucinous	1 (10)	1 (10)	0 (0)	1 (12.5)	1 (10)	0 (0)
poorly diff., serous	0 (0)	1 (10)	0 (0)	0 (0)	4 (40)	2 (15)
unspecified	4 (40)	2 (20)	1 (17)	2 (25)	2 (20)	8 (62)
TOTAL	10 (100)	10 (100)	6 (100)	8 (100)	10 (100)	13 (100)

Fig. 4 Human ovarian xenograft panels for the six data points (drugs) used in the "Ovary *versus* Ovary" relationship in Fig. 3*A*. *A*, names and histology/grade (? = unknown, mod. diff. = moderately differentiated, poorly diff. = poorly differentiated, dediff. = dedifferentiated, adncrc = adenocarcinoma) of all of the xenografts tested. Inclusion of a particular xenograft in one of the panels is shown by a "+" sign in the corresponding row and under the appropriate drug column. *B*, histology/grade subtypes in the human ovarian xenograft panels by number and percentage.

previously treated patients with ovarian cancer were found. Five and one of these compounds had been tested in a panel of 15 and 6 human ovarian xenografts, respectively (26, 30), which fitted the histology/grade patterns identified in Fig. 4*B*. Fig. 6*A* lists the names and Phase II RRs (31–56) of these additional drugs together with the six compounds that were included in the analysis in Fig. 3*A*. Fig. 6, *A* and *B*, also shows mean T/C% values scatter plots and statistical analyses for two cases: first, for when all of the available xenograft information was used, and second, for when mean T/C% calculations were based, where possible, on the arithmetically smallest panel, namely the one used for gemcitabine in Fig. 4. Highly significant correlations were obtained in both cases (Fig. 6*B*).

For NSCLC information on two additional agents was found: amsacrine [mean T/C% of 62 (26) and Phase II RR equal to 0.06 (31)] and doxorubicin [mean T/C% of 47 (26) and Phase II RR equal to 0.12 (32)]. Both had been tested in NSCLC human xenograft panels that included all three histological subtypes and had adenocarcinoma contents of 29 and 33%, respectively. As for ovarian cancer, those two additional data points (Fig. 5*C*, *arrows*) enhanced the statistical significance of the relationship observed in Fig. 3*A*.

DISCUSSION

A literature-based, retrospective study was conducted to examine the clinical predictive value of three widely used preclinical cancer models, namely, the *in vitro* human tumor cell line, the human xenograft, and the murine allograft models. Four solid tumor types were selected, breast, NSCLC, ovary and colon, and data on a set of 31 anticancer agents (excluding agents with novel targets such as signal transduction or angiogenesis modulators) were collected. Preclinical activity in each model was correlated with RRs in Phase II clinical trials by tumor type (disease-oriented approach) in the case when one preclinical tumor type was used as a predictor of overall clinical activity in the other three tumor types combined (compound-oriented approach) and for all four tumor types together.

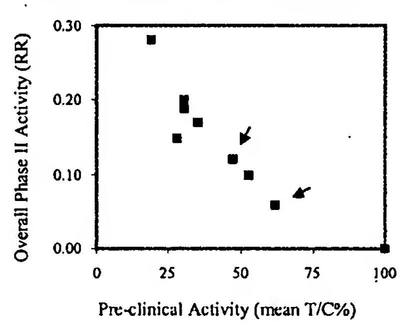
A.

XEN. NAME	XENOGRAFT HISTOLOGY	DRUGS						
		EPI	FAZ	GEM	IRINO	PACLIT	TOPO	VINRLB
T222	squamous cell	+						
T291	adenocarcinoma	+						
UCLA-P3	adenocarcinoma		+					
ACCOLU-78	squamouse cell		+					
NCI-H460	large cell			+	+	+	+	+
A549	adenocarcinoma			+	+	+	+	+
CaLu-6	adenocarcinoma			+				
H-74	?			+				
LC-376	?			+				
QG-56	squamous cell				+			+
NCI-H23	adenocarcinoma				+	+		
NCI-H226	squamous cell				+	+	+	
MV-522	adenocarcinoma					+		
CaLu-3	adenocarcinoma					+		
1° NSCLC	adenocarcinoma					+		
L2987	adenocarcinoma					+		
L-27	adenocarcinoma							+
LC-06	large cell							+
LU-65	large cell							+
PC-12	adenocarcinoma							+
LU-99	large cell							+
TOTAL NO.		2	2	5	5	8	3	8

B.

HISTOLOGY FREQUENCY IN HUMAN NSCLC XENOGRAFT PANELS							
HISTOLOGY	EPI NO. (%)	FAZ NO. (%)	GEM NO. (%)	IRINO NO. (%)	PACLIT NO. (%)	TOPO NO. (%)	VINORLB NO. (%)
adenocarcinoma	1 (50)	1 (50)	2 (40)	2 (40)	6 (75)	1 (33.3)	3 (37.5)
large cell	0 (0)	0 (0)	1 (20)	1 (20)	1 (12.5)	1 (33.3)	4 (50)
squamous cell	1 (50)	1 (50)	0 (0)	2 (40)	1 (12.5)	1 (33.3)	1 (12.5)
unknown			2 (40)				
TOTAL	2 (100)	2 (100)	5 (100)	5 (100)	8 (100)	3 (100)	8 (100)

C. NSCLC VS NSCLC (ADDITIONAL DATA)



r = -0.90 p = 0.001*

Fig. 5 Human NSCLC xenograft panels for the seven data points (drugs) used in the NSCLC *versus* NSCLC relationship in Fig. 3*A*. *A*, drug names (EPI = epirubicin, FAZ = fazarabine, GEM = gemcitabine, IRINO = irinotecan, PACLIT = paclitaxel, TOPO = topotecan, VINRLB = vinorelbine) and histological subtypes (? = unknown) of all of the xenografts tested. Inclusion of a particular xenograft in one of the panels is shown by a "+" sign in the corresponding row and under the appropriate drug column. *B*, histological subtypes in the human NSCLC xenograft panels by number and percentage. *C*, scatter plot and correlation analysis for the same tumor clinical *versus* preclinical activity relationship in NSCLC, including the seven drugs in Fig. 6*A* as well as two additional agents, doxorubicin and amsacrine (data points shown with *arrows*), with known NSCLC Phase II and human xenograft activities.

Colon cancer was the only site for which a disproportional amount of clinically active *versus* inactive agents were identified: only 3 drugs with Phase II RRs > 0.15 and 8 with ≤0.10 (Figs. 1–3). However, this was likely a reflection of the lack of clinically effective drugs for this tumor type rather than the result of selection and publication bias.

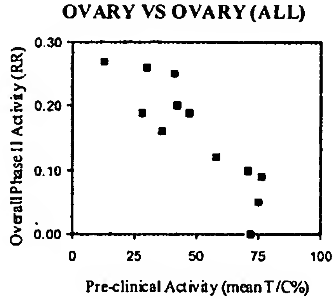
When the mean $Log_{10}GI_{50}$ measure of preclinical activity was used, the *in vitro* cell line model was found to be predictive of Phase II clinical performance for NSCLC under the disease-oriented approach in breast and ovarian cancers under the compound-oriented approach and in the case of all four tumor types together. Highly significant correlations were observed in all cases, except colon cancer, when three consistent outlier data points corresponding to the mechanistically nontypical cytotoxic agents didemnin B, elsamitrucin, and rhizoxin were excluded in exploratory analysis. Thus, the *in vitro* cell line model

A.

	DRUG	PHASE II RESPONSE RATE	HUMAN OVARIAN XENOGRAFT MEAN T/C%	
			ALL TESTED	GEMCITABINE PANEL
STUDY DRUGS	EPIRUBICIN	0.20	42	-
	FOSQUIDONE	0.00	72	-
	GEMCITABINE	0.16	36	36
	MENOGARIL	0.12	58	-
	PACLITAXEL	0.26	30	-
	TAXOTERE	0.27	13	-
ADDITIONAL DRUGS	DOXORUBICIN	0.19 [33-36]	47 [30]	47 [30]
	AMSACRINE	0.05 [31]	75 [26]	-
	CISPLATIN	0.25 [37,38]	41 [30]	46 [30]
	HEXAMETHYL-MELAMINE	0.19 [39-44]	28 [30]	31 [30]
	METHOTREXATE	0.09 [45,46]	76 [30]	84 [30]
	5-FU	0.10 [47-66]	71 [30]	71 [30]

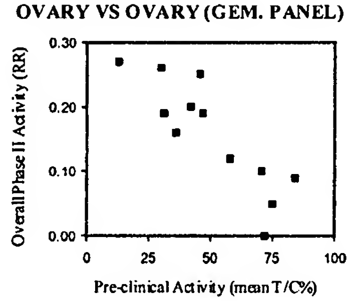
B.

OVARY VS OVARY (ALL)



r= -0.86 p= 0.0004*

OVARY VS OVARY (GEM. PANEL)



r= -0.88 p= 0.0002*

Fig. 6 *A*, preclinical and Phase II clinical activity data for ovarian cancer, including the six drugs in Fig. 3*A* ("Study Drugs") as well as an additional six drugs ("Additional Drugs") with known ovarian Phase II and human xenograft activities. Literature references are shown in superscript font. *B*, scatter plots and correlation analysis for the same tumor clinical *versus* preclinical activity relationship in ovarian cancer based on the data in Fig. 5*A*. Analysis was done for (*a*) when all of the xenografts were included in preclinical activity calculations ("All") and (*b*) when only the six xenografts in the gemcitabine panel were used for preclinical activity calculations, where possible ("Gem. Panel"). Stars indicate statistical significance at the 5% level.

might be predictive in the case of typical cytotoxic cancer agents but might fail to provide reliable information for at least some of the noncytotoxic cancer drugs. Additional studies are needed to explore this observation.

The fact that drug potency (mean $Log_{10}GI_{50}$), a pharmacological measure, was found to be predictive of Phase II performance was somewhat surprising but has been noted previously: a recent study by Johnson *et al.* (18) demonstrated a highly significant correlation between potency in the NCI human tumor cell line screen and activity in the hollow fiber assay. Pharmacological considerations (pharmacological differences between the species) might provide a possible explanation why some anticancer agents appear effective in *in vivo* mouse models but fail to show efficacy in Phase II trials. Experience with some agents (57) has shown that the maximum-tolerated dose in mouse can be higher than in humans, presumably because of an intrinsic ability of mouse cells to tolerate higher drug doses and/or more efficient elimination in the mouse.

In contrast to the *in vitro* cell line, our results suggest that the murine allograft model, as used in this analysis, is not predictive of clinical Phase II performance. This is in agreement with the conclusions from a large body of information originating from the NCI screening programs in use from 1975 to 1990 (5–8, 10–12).

The human xenograft model showed good tumor-specific predictive value for NSCLC and ovarian cancers when panels of xenografts were used. However, it failed to adequately predict clinical performance both in the disease and compound-oriented settings for breast and colon tumors. The results with breast cancer were in agreement with a recent study (18) but were contradictory to the work reported by Bailey *et al.* (20), Inoue *et al.* (21), and Mattern *et al.* (24). However, given that the latter studies did not use formal statistical methods, our conclusions may be more robust. The results for ovarian cancer were in agreement with studies by Taetle *et al.* (23) and Mattern *et al.* (24) but contradicted the conclusions of the recent NCI United States study by Johnson *et al.* (18). Our results for NSCLC were consistent with the observations from all previous studies that examined same tumor correlations in this cancer type (18, 24).

For NSCLC and ovarian cancer patients, a panel of xenografts was more predictive than single xenografts confirming preliminary observations by Bellet *et al.* (19).

In an effort to identify the properties that may render an ovarian or NSCLC human xenograft panel predictive of Phase II drug performance, common characteristics were sought. There was no similarity in number and only limited overlap in identity of xenografts between same tumor type panels. However, certain patterns in histology/grade content were found. These ob-

servations suggest that the relative histology/grade content rather than the number or identity of xenografts within a panel may be the important determinant of clinical predictivity. To our knowledge, no other study has attempted to identify ovarian or NSCLC human xenograft panel features that might lead to accurate predictions of a drug's Phase II performance.

This is the only study that has examined the clinical predictive value of three preclinical cancer models together and thus allows for direct comparisons between them. The results suggest that the human xenograft model is more predictive than its murine allograft counterpart and that the *in vitro* cell line model is of, at least, equivalent usefulness to the human xenograft model.

The NCI work with cancer drug screening programs from 1955 to 1990 (Refs. 5–8, 10–12; leukemia-based preclinical, compound-oriented screens preferentially yielding compounds active against hematological malignancies) in combination with our work and recent conclusions by Johnson *et al.* (Ref. 18; statistically significant results under the compound-oriented approach for some solid tumors) suggest that the compound-oriented strategy may be successful when used only within solid tumors or only within hematological malignancies but not when the two disease groups are considered together.

In general, our results suggest that the *in vitro* human tumor cell line and the human xenograft models might have good clinical predictive value in some solid tumors (such as ovary and NSCLC) under both the disease and compound-oriented strategies, as long as an appropriate panel of tumors is used in preclinical testing.

In conclusion, given the results in this study and those of others (6, 7, 10–12), continued use of the murine allograft model in drug development may not be justified. The work presented here argues for emphasis to be placed on *in vitro* cell lines (in the context of the NCI Human Tumor Cell Line Screen) and appropriate panels of the human xenograft model.

Recent years have seen an explosion in the molecular understanding of cancer, which has led to the development of not only more effective cytotoxic cancer drugs but of potentially cytostatic or antimetastatic agents as well. The future preclinical and clinical development of traditional cytotoxic compounds will likely follow similar procedures with those practiced today, and in that sense, the present findings could contribute to the more efficient discovery of such agents. However, the existing cancer models and parameters of activity in both the preclinical and clinical settings may have to be redesigned to fit the mode of action of the novel cytostatic, antimetastatic, antiangiogenesis, or immune response-modulating agents (58). In the preclinical cancer model front, the case is being made for the use of the orthotopic mouse xenograft and transgenic models (59–61) because those are thought to more accurately simulate human disease, especially in terms of growth characteristics and metastatic behavior. New end points of preclinical activity are contemplated such as the demonstration that a new molecule truly hits the intended molecular target (58). In Phase II clinical trials, there is a growing effort toward validating new surrogate endpoints of drug efficacy (58). The next decade will probably answer many of the questions regarding the effectiveness of these novel agents and will likely define a new role for traditional cytotoxic therapies, but it will also bring new challenges in terms of preclinical predictors of activity.

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DTP Human Tumor Cell Line Screen

Process

The In Vitro Cell Line Screening Project (IVCLSP) is a dedicated service providing direct support to the DTP anticancer drug discovery program. The in vitro cell line screen was implemented in fully operational form in April of 1990. It required approximately five years (1985 - 1990) to develop, and persistence in the effort reflected dissatisfaction with the performance of prior in vivo primary screens. This project is designed to screen up to 3,000 compounds per year for potential anticancer activity. The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The aim is to prioritize for further evaluation, synthetic compounds or natural product samples showing selective growth inhibition or cell killing of particular tumor cell lines. This screen is unique in that the complexity of a 60 cell line dose response produced by a given compound results in a biological response pattern which can be utilized in pattern recognition algorithms (COMPARE program. See: http://dtp.nci.nih.gov/docs/compare/compare.html). Using these algorithms, it is possible to assign a putative mechanism of action to a test compound, or to determine that the response pattern is unique and not similar to that of any of the standard prototype compounds included in the NCI database (see DTP Overview tab). In addition, following characterization of various cellular molecular targets in the 60 cell lines, it may be possible to select compounds most likely to interact with a specific molecular target.

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 uM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels.

Methodology Of The In Vitro Cancer Screen

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid

is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

Publications

Alley, M.C., Scudiero, D.A., Monks, P.A., Hursey, M. L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., and Boyd, M.R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. Cancer Research 48: 589-601, 1988.

Grever, M.R., Schepartz, S.A., and Chabner, B.A. The National Cancer Institute: Cancer Drug Discovery and Development Program. Seminars in Oncology, Vol. 19, No. 6, pp 622-638, 1992.

Boyd, M.R., and Paull, K.D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. Drug Development Research 34: 91-109, 1995.

Cell Lines In The Screen

Please note the links for more information on the SNB-19, U251, NCI/ADR-RES, and MDA-MB-435 cell lines.

- MDA-MB-435
- U251
- SNB-19
- NCI/ADR-RES

Submitting Compounds For Testing In The Screen

Screening Results

Databases of Selected Results

- The Standard AntiCancer Agent Database
- AntiCancer Agent Mechanism Database

- Search All Publically Available Compounds by NSC number